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Effects of Small Intestinal Starch Digestion and Dietary Lipid on Efficiency of Nitrogen Use in Cattle

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EFFECTS OF SMALL INTESTINAL STARCH DIGESTION AND DIETARY LIPID ON EFFICIENCY OF NITROGEN USE IN CATTLE

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Animal Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

AA	amino acid
ADG	average daily gain
ATP	adenosine triphosphate
BW	body weight
C	Celsius
CP	crude protein
Cr	chromium
CrEDTA	chromium ethylenediaminetetraacetic acid
d	day
DIP	degradable intake protein
DM	dry matter
DMI	dry matter intake
DNA	deoxyribonucleic acid
EBW	empty body weight
FA	fatty acid
g	gram
GC	gas chromatography
Glu	glutamic acid
h	hour
H_2O	water
H_2SO_4	sulfuric acid
HCl	hydrochloric acid

HCW	hot carcass weight
i.d.	inner diameter
IU	International units
kg	kilogram
L	liter
LCFA	long chain fatty acid
LISD	large intestinal starch digestion
Lys	lysine
m	meter
M	molar
mbar	millibar
Mcal	megacalorie
MCP	microbial crude protein
ME	metabolizable energy
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mM	millimolar
MP	metabolizable protein
N	nitrogen
NaOH	sodium hydroxide
NDF	neutral detergent fiber

NE_m	net energy for maintenance
NPN	non-protein nitrogen
OM	organic matter
ppm	parts per million
PUN	plasma urea-nitrogen
SEM	standard error of the mean
SISD	small intestinal starch digestion
TiO_2	titanium dioxide
UIP	undegradable intake protein
USP	United States Pharmacopeia
UT-B	urea transporter-B
VFA	volatile fatty acid
vol	volume
WDGS	wet distillers' grains with solubles
wt	weight

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ABSTRACT

EFFECTS OF SMALL INTESTINAL STARCH DIGESTION AND DIETARY LIPID ON EFFICIENCY OF NITROGEN USE IN CATTLE

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The objective of this research was to determine the effects of increases in energy available for gain from increased small intestinal starch digestion (SISD) and dietary lipid source and amount on the efficiency of N use in cattle. Five ruminally, duodenally, and ileally cannulated steers were placed in a 5 × 5 Latin square. Each received duodenal infusion of 1.5 ± 0.08 kg/d raw cornstarch and either 0, 30.9 ± 0.59, 62.4 ± 1.16, or 120.4 ± 3.39 g/d Glu, or 387.9 ± 17.47 g/d casein. Casein increased ($P = 0.05$) SISD. Similarly, greater duodenal Glu linearly ($P = 0.02$) increased SISD. Starch flow to the ileum decreased (*Linear* = 0.04) in response to greater postruminal Glu. Ileal flow of ethanol-soluble starch was not affected by duodenal Glu (*Linear* = 0.16) or casein ($P = 0.42$). Fecal starch flow was decreased by Glu (*Linear* = 0.04) and casein ($P = 0.01$), thus increasing postruminal starch digestion in response to casein ($P = 0.02$) and Glu (*Linear* = 0.05). Urinary N excretion was not affected ($P \geq 0.30$) by postruminal Glu flow but urine N was increased by casein ($P < 0.01$). Nitrogen balance was not affected by greater duodenal Glu ($P \geq 0.34$) despite increases in SISD, but casein increased N retention ($P < 0.01$). Duodenal casein ($P = 0.38$) and increasing amounts of duodenal Glu ($P \geq 0.15$) had no effect on N retained as a proportion of N intake. The same steers were fed corn-based diets with varying amounts and sources of lipid in a 5 × 5 Latin square. Diets contained no supplemental fat (**CON**), 4% supplemental saturated fat (tallow, **4S**), 4%

supplemental unsaturated fat (linseed oil, **4U**), 8% supplemental saturated fat (tallow, **8S**), or 8% supplemental unsaturated fat (linseed oil, **8U**). Increasing level of lipid supplementation did not affect DMI; however, unsaturated lipid reduced ($P = 0.05$) DMI. Apparent ruminal OM digestibility tended (*Linear* = 0.08) to decrease with increasing dietary lipid. Total-tract digestibility of DM (*Linear* = 0.07), OM (*Linear* = 0.11), and NDF (*Linear* = 0.11) also tended to decrease in response to greater dietary lipid. Additionally, unsaturated lipid tended ($P = 0.07$) to reduce total-tract NDF digestibility compared to saturated lipid. Ruminal pH was not affected by treatment ($P \geq 0.35$). Greater dietary lipid did not affect ($P \geq 0.30$) total organic acid production; however, ruminal acetate (*Linear* = 0.05) and the ratio of acetate:propionate were decreased. Interestingly, ruminal acetate ($P = 0.07$), propionate ($P = 0.06$), and the ratio of acetate:propionate ($P = 0.02$) was affected by the interaction of source × level. Duodenal flow of microbial N was not different ($P \geq 0.17$). Microbial efficiency was linearly ($P = 0.05$) increased with increased level of dietary lipid. Unsaturated lipid decreased urinary N ($P = 0.02$) and fecal N ($P < 0.01$). Despite altered urine and fecal N outputs, small variations in N intake, urinary N, and fecal N mitigated responses among N balance. N efficiency was not affected ($P \geq 0.18$) by lipid source or amount.

CHAPTER ONE:

INTRODUCTION AND LITERATURE REVIEW

Introduction

Cattle use N from dietary protein and non-protein N sources to synthesize amino acids for productive purposes such as meat or milk production and body protein turnover. This process of converting lesser quality plant proteins and nitrogenous compounds into high-quality animal protein is complex and possibilities for improvements in the efficiency of N utilization exist. The efficiency with which cattle utilize N can be variable based on the type of production it is supporting and is generally poor in comparison to their nonruminant counterparts. Factors such as N recycling in the form of urea, dietary energy as starch or lipid, improvements in small intestinal starch digestion, and growth promoting technologies aid in the improvement of N efficiency in cattle. Numerous research has been performed in attempt to improve the efficiency of N utilization in cattle with management or feeding strategies; however, more research is warranted. Ultimately, improving the efficiency of N utilization in cattle may provide greater production benefits to cattle production systems. We hypothesized that altering small intestinal starch digestion or dietary fat content could affect efficiency with which cattle utilize N.

Literature review

Nitrogen metabolism in cattle

Dietary N in cattle diets is derived from true proteins (e.g., plant or animal protein) or as non-protein nitrogen (NPN; e.g., urea). The 'crude' protein system classifies dietary N as either degradable (degradable intake protein; DIP) or undegradable (undegradable intake protein; UIP) by ruminal microbes. Generally, DIP is degraded by ruminal proteases, peptidases, and deaminases produced by the ruminal microbiota. These microbial enzymes are responsible for the production of AA and ammonia required for microbial crude protein (MCP) synthesis. Typically, ruminal microbiota produce ammonia via deamination of AA that exceed requirements for microbial growth, and when DIP exceeds fermentable energy available for microbial growth ammonia can accumulate (Wallace, 1996).

Ammonia N is able to be recycled for subsequent use in microbial protein synthesis. This recycling of N may allow opportunity for improved efficiency of N utilization in cattle. Excess ammonia can move across the rumen wall to enter the blood stream via passive diffusion; however, in neutral or acidic environments ($pH < 9.2$) ammonia is converted to ammonium which requires active transport for absorption to blood (Abdoun et al., 2007). Ammonium requires conversion to ammonia at the gut wall before it can enter into the epithelial cell, then it is re-protonated to form ammonium (Huntington and Archibeque, 2000).

Nearly all (70-95%) portal ammonia arriving at the liver is extracted and converted by hepatic tissues to urea or Gln (Parker et al., 1995). Total capacity for ureagenesis is not typically exceeded in the liver and is closely related to dietary N

amounts and the quantity of ammonia absorbed from the digestive tract (Huntington and Archibeque, 2000).

Hepatic tissues require a considerable amount of energy to carry out ureagenesis. Production of one mole of urea by the liver requires 4 ATP; however, a considerable amount of energy can be generated from the process of ureagenesis. Waterlow (1999) reported that 6 moles of ATP can be generated from the production of 1 mole of urea, which actually exceeds the energy expended. Indeed, Firkins and Reynolds (2005) concluded that energetic costs of ureagenesis were not large.

About 40 to 80% of urea synthesized hepatically can reenter the digestive tract at any segment along the tract (Siddons et al., 1985; Lapierre and Lobley, 2001). Postruminal tissues may accept a large portion of the urea that enters the gut, but most microbial protein synthesized from this urea is likely expelled in the feces (Lapierre and Lobley, 2001). Urea recycled to the rumen allows opportunity for urea-N to be incorporated to AA produced by ruminal microbiota, and the rumen does seem to be a major sink for hepatically synthesized urea.

Ruminants rely heavily on urea entry into the gut for production of microbial protein to buffer variations in dietary supply of N (Reynolds and Kristensen, 2008). Transport of urea into the rumen depends largely on urea transporters (Abdoun et al., 2010). Urea transport-B (UT-B) proteins are largely responsible for mediating transfer of urea down a concentration gradient from the blood into the rumen (Walpole et al., 2015). Abdoun et al. (2010) demonstrated that ruminal urea transport rates of UT-B could be readily increased by the presence of VFA or CO_2 at a typical ruminal pH range of about 6.4 compared to a more neutral pH of 7.4. The ruminal uptake of urea is often greatest 30

to 90 min postprandial (Rémond et al., 2003), and Abdoun et al. (2010) further suggested that the influx of urea via UT-B is closely linked to microbial demand for N, which is typically greatest postprandially and is characterized by conditions of decreased pH and VFA production. Circulating urea can also enter the digestive tract through saliva.

Endogenously produced urea entering the rumen can arrive via saliva; however, amounts vary based on forage concentration (Lapierre and Lobley, 2001). Huntington (1989) reported that 69% of ruminal urea entry was derived from saliva among steers fed alfalfa hay, but only 23% in steers fed a cracked corn-based diet. Huntington (1989) concluded that differences were likely attributable to decreased saliva production and urea concentration with reduced diet forage content. In steers fed alfalfa hay and infused with ruminal or abomasal combinations of casein and cornstarch, Taniguchi et al. (1995) reported 36% of endogenous urea entered the gut via saliva. Fortunately for cattle, the ability to shuttle blood urea to the digestive tract through transfer across the rumen epithelium and also via saliva entry allows greater utilization of N, especially through increased microbial protein synthesis in the rumen (Lapierre and Lobley, 2001).

Microbial urease activity liberates ammonia from the endogenous urea than enters the rumen for microbial protein synthesis. The MCP flowing out of the rumen is made up of approximately 80% true protein, and protein is typically 80% digestible in the small intestine (NRC, 2001). Thus, the NRC (2001) predicts that 64% of MCP is metabolizable protein. All of the undegraded feed protein is assumed to be true protein, but the digestibility of this UIP is varied between feedstuffs (NRC, 2001).

Postruminal digestion of protein begins in the abomasum, where secretion of acid, pepsin, and lysozymes occurs. These secretions work to lower pH to around 2, cleave

peptide bonds, and lyse microbial cells which make up a large proportion of the protein flowing out of the rumen. Once digesta passes out of the abomasum, it is subjected to pancreatic secretions that neutralize chyme and hydrolyze protein. The small intestine absorbs end products of protein hydrolysis (e.g., small peptides, AA) via mucosal transport proteins (Asplund, 1994). Once absorbed, AA are available for protein synthesis.

Factors affecting N efficiency

Efficiency of N utilization can be variable in cattle depending on a variety of factors including diet composition, previous plane of nutrition, and management practices. Nitrogen efficiency typically ranges between 30 and 50% in cattle. Bierman et al. (1999) measured N balance in steers fed treatment diets of varying fiber levels and observed N efficiencies that ranged from 33.77 to 49.26%. When Waldrip et al. (2013) analyzed 12 different individual feeding and N flux studies, they calculated that average N efficiency was 26.54%. In yearling cattle fed starch-based diets, Erickson and Klopfenstein (2001) measured N intake and retention and reported N efficiencies ranging between 10.87 and 14.03% of N intake.

Spanghero and Kowalski (1997) reviewed the available literature on N balance in dairy cows and reported that 34.15% of diet N was used for productive purposes (i.e., milk or tissue production) during early to mid-lactation. Clearly, the efficiency with which cattle utilize N is less than the efficiency of nonruminants.

Ball et al. (2013) observed N efficiencies that ranged from 52 to 64% when they fed pigs different concentrations of dietary CP and available Lys. Similarly, Andretta et

al. (2016) measured N balance in pigs and reported efficiency of N use values ranging from 44 to 56%. Rand et al. (2003) reviewed the available literature and found the median N efficiency in man to be about 47%. The N efficiency of both ruminants and nonruminants is dependent on many variables.

Transfer of N between the circulatory system and digestive tract, dietary CP concentrations (Archibeque et al., 2007), and MP flow to the small intestine (Archibeque et al., 2008; Hales et al., 2013) can alter N efficiency in cattle. Further, dietary energy from starch or fat (Reynolds et al., 2001), increases in glucose absorption from small intestinal starch digestion (**SISD**) (Firkins and Reynolds, 2005), and growth promoting technologies can also impact the efficiency of dietary N for productive purposes. A more comprehensive understanding of the roles of these factors towards improving N efficiency could provide a large benefit to cattle production.

Transfers of N between blood and gut

All cattle produce urea in the liver and kidney from ammonia; however, endogenous production of urea cannot augment efficiency of N utilization in ruminants if endogenously produced urea does not transit from the blood to segments of the alimentary tract that contain microbiota that can synthesize AA from urea-N.

Apparently, ruminants have large capacity to recycle N from plasma urea to the rumen. Siddons et al. (1985) reported that 27 to 44% of the plasma urea returned to the digestive tract was returned to the rumen of sheep. Lapierre and Lobley (2001) determined that about 50% of the urea entering the gut (i.e., around one third of total hepatic urea production) enters the rumen of cattle where it can be converted to amino

acid via microbial synthesis. Microbial synthesis of AA from endogenous urea-N allows for greater efficiency of N use by salvaging an end product of catabolism (i.e., plasma urea) into a productive product (i.e., amino acids) for anabolism. Indeed, as much as 23 (Bailey et al., 2012b), or 25% (Brake et al., 2010) of microbial N flowing to the duodenum of cattle can originate from recycled N.

A large amount of endogenously produced urea (up to 70% of total urea production) can enter the gastrointestinal tract at sites other than the rumen (e.g., small and large intestines); however, gut entry of urea at these sites does not contribute to anabolic production (Lapierre and Lobley, 2001). The small intestine is the primary site of amino acid absorption, but the ability for urea or ammonia to be incorporated into AA via microbial synthesis is minimal. The inverse is true of the large intestine, where urea that enters the gut can readily be synthesized into AA by a much larger population of microbes, but the absence of mechanisms for AA uptake cause nearly all these AA to be excreted in the feces.

Dietary energy as starch

Production by cattle is often most limited by energy. Therefore, non-grazing cattle are often fed starch-based diets that contain large amounts of cereal grains (e.g., corn, barley, sorghum). Feedlot cattle often consume greater than 5,000 g of starch daily (Theurer, 1986). In the United States, corn, which is typically composed of about 72% starch (Huntington et al., 2006), provides the majority of dietary starch fed to feedlot cattle (Vasconcelos and Galyean, 2007). Dietary starch content can impact the amount of

N needed among ruminal bacteria and greater amounts of net energy from diet can augment production in cattle that can allow greater N efficiency in cattle.

The rumen is often the primary site of starch digestion among cattle fed starch-based diets. Starch granules from corn are composed of both amylose (glucose molecules linked by α-1,4 bonds) and amylopectin (glucose molecules linked by α-1,4 bonds and highly branched with α-1,6 bonds). These polysaccharides are predominantly hydrolyzed by amylases produced by the amylolytic population of bacteria in the rumen during fermentation (Kotarski et al., 1992). However, protozoa and fungi also play a smaller role in ruminal starch digestion (McAllister et al., 1994). The amount of dietary starch presented to the rumen microbes can influence the efficiency of N use within the rumen.

Dietary starch content can affect the amount of N available to the rumen microbes. Ammonia is the predominant source of N for ruminal bacteria (Russell et al., 1992), and its ruminal concentration is dictated by ruminally available N and energy available for microbial growth (Hristov and Jouany, 2005). It has been well documented that the starch (or glucose) supplementation to the rumen decreases ruminal ammonia concentration in dairy (Rooke et al., 1987; Cameron et al., 1991; Hristov et al., 2005; Fredin et al., 2015) and beef cattle (Taniguchi et al., 1995; Bailey et al., 2012a). Greater fermentable energy supply to ruminal microbes can augment microbial growth and increase incorporation of ruminally available N to microbial AA. Indeed, Bailey et al. (2012b) reported increased microbial N from recycled urea in cattle receiving ruminal glucose infusions compared to control. Efficiency of N use is improved when ruminally available N is incorporated into microbial mass rather than absorbed into blood as ammonia and excreted in urine as urea (Hristov and Jouany, 2005). Furthermore, greater

fermentable energy supplies increase utilization of ruminally available N and allow for increased microbial protein synthesis.

Hristov et al. (2005) reported that microbial N flows to the duodenum were increased more than 20% among cattle supplemented with glucose or cornstarch compared to oat fiber supplementation. Further, these authors (Hristov et al, 2005) observed a 25% increase in microbial efficiency among cattle provided glucose or starch compared to oat fiber; however, when Bailey et al. (2012a) ruminally administered 1,200 g/d glucose into the rumen of cattle consuming forage-based diets, they reported no change in flows of microbial N and a decrease in the efficiency of microbial N synthesis. Similarly, Fredin et al. (2015) found no differences in microbial N flows of cattle fed greater amounts of starch (28.5% diet DM compared to 22.8% diet DM).

Dietary energy as lipid

Lipid is often added to cattle diets to increase energy density (Vasconcelos and Galyean, 2007); however, dietary fat can have a profound impact on the rumen environment. Antimicrobial effects of lipids can limit fermentation and digestion of feed (Jenkins, 1993). Inhibitory effects of lipid have been reported in both forage- (Elliott et al., 1997; Oldick and Firkins, 2000) and starch-based (Plascencia et al., 2012) diets. Elliott et al. (1997) reported that ruminal OM truly digested was reduced among cattle fed forage-based diets that contained up to 5.7% lipid (DM-basis), and others (Oldick and Firkins, 2000) observed decreased ruminal NDF digestibility among cattle fed corn silage-based diets containing similar amounts of fat. Plascencia et al. (2012) supplemented yellow grease to steers fed corn-based diets and measured an 11%

reduction in ruminal OM digestibility. Typically, decreases in ruminal OM digestibility among cattle fed starch-based diets can be accounted for by decreased digestibility of structural carbohydrates (e.g., cellulose, hemicellulose), because starch digestion is often not impacted by dietary lipid content (Jenkins, 1993; Zinn et al., 1994).

Increased dietary lipid content can have mixed effects on duodenal flow of microbial N (Oldick and Firkins, 2000; Gozho et al., 2008; Mutsvangwa et al., 2012). Thus, the microbial efficiency (g microbial N/kg OM truly fermented) can be unaffected (Avila et al., 2000; Oldick and Firkins, 2000) or even increased (Górka et al., 2015) by increasing lipid supplementation. However, data are limited on effects of diet lipid content on N balance in cattle. Gozho et al. (2008) reported no change in tissue N retention (30.9 g/d) or milk N (152.0 g/d) among dairy cattle fed diets containing up to 5.3% lipids (DM-basis) varying in degree of unsaturation; however, these authors did not quantify effects of added lipid. Benchaar et al. (2015) fed silage-based diets containing 6% fat (provided from linseed oil) and observed no change in tissue N balance in dairy cattle, but milk N tended ($P = 0.07$) to decrease, therefore these authors reported no change in productive N (N intake – total N excretion). These authors (Benchaar et al., 2015) found no difference in the efficiency of tissue N, milk N, or productive N utilization as a proportion of N intake. More integrated information is clearly needed to develop a more precise understanding of the effects of supplementary lipid on efficiency of N use in growing cattle.

Dietary protein

Dietary protein intake can impact efficiency of N utilization in cattle, because amounts of N excretion can be affected by amounts of dietary N (Satter et al., 2002). Hales et al. (2013) fed 8 Jersey steers a steam-flaked corn-based diet with increasing amounts of CP from wet distillers' grains with solubles (WDGS) and found no effect of WDGS inclusion on N retention (37.2 ± 11.56 g/d; *Linear* = 0.81) or efficiency of N use (22.9 ± 7.29%; *Linear* = 0.54). However, Bailey et al. (2012a) observed greater N balance and efficiency of N utilization when casein was ruminally infused in the rumen of cattle fed a forage-based diet.

When Huhtanen and Hristov (2009) reviewed the available literature, they concluded that reduced N intake had the greatest impact on amounts of N excreted and efficiency of N utilization among dairy cattle. Sinclair et al. (2014) concluded that milk N efficiency was most effectively increased by reducing N intake, because protein is typically overfed to dairy cattle in the US. Interestingly, the proportion of N that is degraded in the rumen does not appear to affect overall N efficiency in cattle. Huhtanen and Hristov (2009) reported that there was no correlation between degradable protein and the efficiency of N utilization, and only dietary CP content was strongly associated to N efficiency.

Apparently, there has been little improvement in efficiency with which cattle use dietary N over the last 50 years. Indeed, Calsamiglia et al. (2010) concluded that the average N efficiency among cattle in 1960 averaged 23.7% and has seemingly not improved since. These studies may indicate that there is a need for a more comprehensive understanding of the factors affecting the efficiency of N use in cattle.

Small intestinal starch digestion

The small intestine is a major site of nutrient absorption in cattle. Typically daily starch intakes can exceed 5 kg among cattle fed starch-based diets (Theurer, 1986), and because ruminal starch digestibility is about 72% (Owens et al., 1986) more than 1,400 g/d of starch can flow to the small intestine. Small intestinal starch digestion is limited among cattle and other ruminants compared to nonruminants that typically digest more than 95% of starch flowing to the duodenum. Owens et al. (1986) calculated that the average SISD among cattle fed starch-based diets was 52.9 ± 18.6%. Starch digested in the small intestine can provide greater energy than ruminally digested starch. Increases in retained energy from SISD compared to ruminal starch fermentation are apparently large and range from 32 (Harmon and McLeod, 2001), or 34 (McLeod et al., 2001) to 42% (Owens et al., 1986). Increasing energy available for gain from greater SISD could provide a greater opportunity for lean tissue deposition and thus, improve N efficiency. Considerable effort has been made to attempt to stimulate SISD in cattle.

Increasing flows of postruminal protein can increase SISD in cattle. Taniguchi et al. (1995) observed greater net glucose release to the portal-drained viscera when cattle were provided greater amounts of casein to the abomasum. Similar increases in SISD have also been reported among cattle receiving increasing amounts of casein to the duodenum (Richards et al., 2002; Brake et al., 2014b). Considerably less effort has been made to measure the effects of greater postruminal flows of individual amino acids on SISD; however, it appears postruminal Glu alone can mimic the effects of casein on SISD in cattle (Brake et al., 2014a).

Taniguchi et al. (1995) reported a nearly 50% increase in retained N and efficiency of N utilization when SISD was increased with greater postruminal casein flows. McLeod et al. (2001) reported that retained N was increased by 32% with abomasal infusion of partially hydrolyzed starch in comparison to water.

Apparently, increased SISD can augment lactation in addition to increases in growth. Reynolds et al. (2001) observed a linear increase in milk yield and milk protein yield in response to greater apparent SISD. Additionally, these authors (Reynolds et al., 2001) reported a 2-fold increase in tissue energy balance, while retaining more than 3-fold more tissue N. Further, efficiency of N utilization for productive purposes (i.e., tissue N and milk N) was increased 25% with greater SISD. These data suggest that greater energy available for productive purposes from improved SISD can improve the efficiency of N utilization in cattle.

Growth promoting technologies

Anabolic implants have been shown to improve ADG, feed efficiency, and the proportion of lean to fat in the carcass (Preston, 1999). Additionally, anabolic implants and can also improve the efficiency of N utilization in cattle. Anabolic implants are placed in more than 90% of cattle placed in feedlots in the United States (USDA, 2011).

Cecava and Hancock (1994) administered estradiol implants to cattle fed isonitrogenous diets and observed decreased urine N, which resulted in a nearly 17% increase in N retained compared to control cattle. This improvement in N balance resulted in an increase in N efficiency (51.5%) compared to the control cattle (43.6%).

Additionally, others (Hunter et al., 1998) reported increased N retention in cattle receiving an anabolic implant, resulting in a 27% increase in N efficiency.

β-adrenergic agonists are synthetic catecholamines that increase muscle mass by increasing the proportion of protein to DNA (Johnson et al., 2014). Thus, β-adrenergic agonists repartition energy from fat accretion towards protein accretion. Increases in lean tissue accretion due to these repartitioning agents can lead to increased N retention in cattle (Walker et al., 2007; Brake et al., 2011). Walker et al. (2007) observed greater N retention in ractopamine fed steers (42.5 g/d) compared to control steers (37.5 g/d) which resulted in a 17% increase in N efficiency. Similarly, Brake et al. (2011) reported a 62% increase in N balance in steers consuming zilpaterol; however, greater N intake among steers consuming zilpaterol obfuscated measures of N retention. Clearly growth promoting technologies such as anabolic implants and β-adrenergic agonists can promote more efficient capture of dietary N.

Previous plane of nutrition

Cattle are able to accelerate their growth proportional to the level of restriction previously experienced after a period of restricted growth followed by a period of realimentation (Hornick et al., 2000). Thus, compensatory gains allow cattle with restricted growth to return to normal growth when fed diets able to support their genetic potential for growth. Cattle are able to deposit body tissues during the compensatory period with greater efficiency than cattle under normal growth conditions. Thus, compensatory gains can impact N efficiency of cattle.

Apparently, when diet restricts growth, nutrient requirements for maintenance are reduced (Hornick et al., 2000). A reduction in visceral mass and metabolism is largely responsible for this decreased basal metabolism.

Cattle with compensatory growth have been reported to deposit a greater proportion as lean tissue rather than fat (Carstens et al., 1991; Keogh et al., 2015). Carstens et al. (1991) reported greater protein accretion on an EBW basis for steers with compensatory growth than control which resulted in greater ADG for cattle with compensatory gains. Similarly, Keogh et al. (2015) measured greater longissimus dorsi growth in addition to greater ADG for cattle with compensatory gains compared to cattle that did not experience restrictions in growth. This accelerated growth can influence the efficiency of N use.

Santra and Pathak (1999) measured N utilization of cattle restricted in growth and observed no change in the N balance of restricted cattle as a proportion of metabolic body size; however, N balance was increased when cattle with restricted growth were realimented. Further, cattle experiencing compensatory growth retained N more efficiently compared to control (Santra and Pathak, 1999). Similarly, when Blum et al. (1985) reported reduced N balance during periods of restricted growth, but increased N balance during periods of compensatory growth. Increased N balance resulted in greater N efficiency as a proportion of N intake (Blum et al, 1985). Clearly, compensatory growth can augment greater efficiency of N use; however, compensatory growth is not sustainable for long periods of time. Maximum growth rates are typically observed around 1 month after realimentation, and generally return to normal after approximately 4 months (Hornick et al., 2000).

Conclusions

In summary, N efficiency in cattle can be variable and is often poor in comparison to nonruminants. Factors such as improved urea recycling to the digestive tract, dietary starch or fat content, dietary protein content, improved SISD, or previous plane of nutrition can have large impacts on N efficiency.

Increased SISD can increase efficiency of N utilization in cattle. A better understanding of mechanisms responsible for SISD may allow for improved diets and management techniques that increase feed efficiency in cattle. Increases in diet lipid content can increase net energy content of diets. Thus, increases in dietary lipid can contribute to increases N use by cattle; however, effects of dietary lipid may be mitigated by decreased ruminal fermentation of feed.

Little progress has been made to improve the overall efficiency of N utilization among cattle despite considerable research efforts. Thus, a need for more integrated information on the factors affecting N efficiency in cattle is warranted and could provide a large benefit to cattle production.

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CHAPTER 2:

INCREASES IN DUODENAL GLUTAMIC ACID FLOW LINEARLY INCREASE SMALL INTESTINAL STARCH DIGESTION BUT NOT N BALANCE IN CATTLE[1]

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ABSTRACT

Small intestinal starch digestion (SISD) in cattle is often limited; however, greater postruminal flow of high-quality protein (e.g., casein) can increase SISD, and Glu can mimic responses in SISD similar to casein. We evaluated effects of increasing Glu flows to the duodenum on SISD and N retention in cattle. Cattle received (DM-basis) continuous duodenal infusion of raw cornstarch (1.5 ± 0.08 kg/d) and either 0, 30.9 ± 0.59, 62.4 ± 1.16, or 120.4 ± 3.39 g/d Glu, or 387.9 ± 17.47 g/d casein. As expected, the positive control (i.e., casein) increased ($P = 0.05$) SISD. Interestingly, SISD increased linearly ($P = 0.02$) with increasing amounts of Glu. Starch flow to the ileum decreased (*Linear* = 0.04) in response to greater postruminal Glu and tended to decrease ($P = 0.07$) with duodenal casein infusion. Ileal flow of ethanol-soluble starch was not affected by duodenal Glu (*Linear* = 0.16) or casein ($P = 0.42$). There was a tendency (*Quadratic* = 0.08) for a curvilinear response among ileal glucose flow to increases in duodenal Glu, but casein had no effect on glucose ($P = 0.81$) flows to the ileum. Greater postruminal flows of Glu (*Linear* = 0.04) and casein ($P = 0.02$) decreased fecal starch flow. Postruminal starch digestion was increased by both casein ($P = 0.03$) and Glu (*Linear* = 0.05). Nitrogen intake from feed was not different ($P \geq 0.23$). By design, infusate N increased from 0 to 13 ± 1.5 g/d with greater amounts of Glu (*Linear* < 0.01), and casein provided 61 ± 1.3 g N/d in comparison to control ($P < 0.01$). Urinary N excretion was not affected ($P \geq 0.30$) by postruminal Glu flow but urine N was increased by casein ($P < 0.01$). Glutamic acid did not affect N retention ($P \geq 0.34$) despite increases in SISD, but casein increased N retention ($P < 0.01$). It is possible that increases in energy available for gain from increased SISD in response to greater postruminal flow of Glu are used for

purposes other than protein deposition. However, it is likely that increases in energy available for gain exceeded capabilities for N deposition under conditions of our experimental model, because N retained as a proportion of N intake (26.7 ± 0.02%) was not different when cattle were provided Glu ($P \geq 0.15$) or casein ($P = 0.38$).

Key Words: cattle, glutamic acid, nitrogen, small intestine, starch digestion

INTRODUCTION

Small intestinal starch digestion (SISD) can provide greater energy than ruminal fermentation of starch (Owens et al., 1986; McLeod et al., 2001); however, SISD can be limited in comparison to ruminal fermentation (Owens et al., 1986; Huntington et al., 2006; Harmon, 2009). Consequently, cattle are typically fed diets with large amounts of ruminally fermentable starch. Unfortunately, increases in ruminal degradation of starch are associated with reduced DMI and increased incidence of metabolic disorders (Owens et al., 1998; Huntington et al., 2006; Krehbiel et al., 2006).

Greater postruminal flows of casein can increase SISD in cattle (Taniguchi et al., 1995; Richards et al., 2002; Brake et al., 2014b). Richards et al. (2002) and Brake et al. (2014b) observed linear increases in SISD when cattle were infused with as much as 200 or 400 g/d of postruminal casein, respectively. Postruminal flows of Glu in amounts similar to that provided by casein mimic increases in SISD to casein (Brake et al., 2014a); however, data are limited on effects of increasing amounts of postruminal Glu on SISD in cattle.

It remains equivocal if increases in energy available for gain from greater SISD can improve N balance in cattle. Reynolds et al. (2001) reported that increases in SISD alone improved N balance among lactating cows; however, McLeod et al. (2001) observed only modest increases in N retention when greater quantities of starch were apparently digested in the small intestine. McLeod et al. (2001) concluded that limitations in N available for lean tissue deposition limited potential increases in N retention, which contributed to greater amounts of energy from increased SISD retained as lipid versus lean tissue. No data are available on N balance in cattle when energy available for gain is

increased by greater SISD in response to duodenal Glu. We hypothesized that increases in postruminal Glu flow would increase SISD and N retention.

MATERIALS AND METHODS

Animals and diets

All experimental protocols and animal husbandry procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

Five Limousin × Jersey steers (initial BW = 351 ± 11.0 kg) were fitted with ruminal cannulas using a one-stage procedure (Kristensen et al., 2010) in addition to duodenal and ileal cannulas (Streeter et al., 1991). Cattle were placed in a 5 × 5 Latin square with 12-d periods. Each period consisted of 8 d for adaptation to treatment, and samples were collected during the final 4 d. Cattle were housed in individual pens (1.7 × 2.4 m) in a temperature controlled room (21°C) under 16 h of light (0500 to 2100 h) and 8 h of darkness. Cattle were fed 5.1 kg/d (DM basis; 0700 and 1900 h) of a soybean hull-based diet (Table 2.1; about 1.5 × maintenance energy requirement; NRC, 2000). The diet was formulated to provide little amounts of starch, but adequate ruminally available N and to meet or exceed requirements for vitamins and minerals (NRC, 2000). The diet was formulated to supply adequate MP to support 0.68 kg ADG.

Infusions

Treatments were continuous duodenal infusions of raw cornstarch (1,507 ± 18 g DM/d) and either 0 (control), 30.9 ± 0.59, 62.4 ± 1.16, or 120.4 ± 3.39 g Glu/d, or 387.9 ± 18.34 g casein (DM/d; a positive control). Infusions of Glu were designed to deliver

37.5, 75, and 150% of amounts of Glu provided by casein. The pH of suspensions containing Glu were adjusted to near 7 with addition of 18.2, 39.4, or 85.6 g NaOH (40% wt/wt), respectively. Duodenal infusions were delivered via a peristaltic pump (model CP-78002-10; Cole-Parmer, Vernon Hills, IL) through Tygon tubing (i.d. = 2.38 mm; Saint Gobain North America, Valley Forge, PA). Each 12-h, cornstarch suspensions (7 L) were prepared by weight and the residual infusate was recorded after each 12-h infusion to determine the mass infused. Suspensions were maintained with continuous stirring by an electric mixer (Arrow 1750, Arrow Engineering Company, Hillside, NJ) and delivered at a rate of 536 mL/h. Cornstarch suspensions (900 g cornstarch; Clintose 106, Archer Daniels Midland Company, Chicago, IL) contained CrEDTA (0.075 g Cr/L, Binnerts et al., 1968) which served as an indigestible marker, and deionized H_2O. To prevent sedimentation of infusate within the infusion line, the cornstarch suspensions were elevated and each infusion line was flushed with 100 mL deionized H_2O every 12 h.

Measurements

Cattle were moved to metabolism crates (0.66 × 1.83 m) on d 8 of each period to allow measures of N retention concurrent with measures of SISD. Diet (100 g/d) and ort samples (10%, if present) were collected from d 8 to 11 to correspond to ileal and fecal samples collected on d 9 to 12. Ileal and fecal spot samples were collected each 4 h between 0700 and 1900 h on d 9 to 12 and composited. Sampling time was delayed 1 h each subsequent d so that composites were representative of each h in a 12-h period. Total urine output was measured on d 9 to 12 of each period by collecting urine from each steer daily into a clean container with 900 mL of 10% (wt/wt) H_2SO_4.

Ileal digesta samples (148 ± 2.7 g) were collected by attaching a plastic bag (140 × 229 mm, Fisherbrand, Fisher Scientific) to the cannula, and fecal samples (166 ± 2.6 g) were collected after manual stimulation of defecation. The pH of ileal samples was immediately measured with a pH meter (Orion 3 Star, Thermo Scientific, Waltham, MA). Fecal pH was measured after mixing 5 g of feces with 15 mL of distilled, deionized H_2O. Subsequently, ileal digesta and feces were alkalinized to obviate innate α-glycohydrolase activity on subsequent measures of starch content by addition of 2.1 mL 40% (wt/wt) NaOH to achieve a final pH of 11.5 ± 0.13 and 9.2 ± 0.18 respectively. Samples were composited and frozen (-20°C) after they were alkalinized.

Jugular blood was collected into heparinized (143 USP) tubes (16 × 100 mm; BD, Franklin Lakes, NJ) via venipuncture 12-h after feeding on d 12. Blood tubes were immediately placed on ice and plasma was harvested after centrifugation (2,200 × g; 15-min; 4°C) and frozen (-20°C).

Prior to analyses, ileal digesta and feces were thawed at room temperature. An aliquot of feces was dried at 55°C and ground to pass a 1-mm screen (Thomas Wiley Laboratory Mill Model 4; Thomas Scientific USA, Swedesboro, NJ) and another aliquot of ileal digesta (75 g) or feces (50 g with 25 mL distilled, deionized H_2O) was neutralized with 6 *M* HCl. Sample DM was determined by drying for 24 h at 105°C (method no. 934.01; AOAC, 2012). Feed, ileal digesta, and feces were analyzed for starch content using the methods of Herrera-Saldana and Huber (1989) followed by a glucose oxidase assay (Gochman and Schmitz, 1972). Unpolymerized glucose was determined from starch assay tubes to which no enzymes were added. Ethanol-soluble starch content was determined using methods similar to those described by Kreikemeier and Harmon (1995).

Briefly, neutralized digesta and feces were centrifuged (20,000 × g; 15-min; 4°C) and 0.5 mL of supernatant was transferred to a microcentrifuge tube (2.0 mL, Safe-Lock Tubes, Eppendorf, Hauppauge, NY) containing 1.25 mL of anhydrous ethanol. Samples were refrigerated (4°C) overnight (16 h) then centrifuged (17,000 × g; 10-min; 4°C) and the supernatant was transferred to a 15 mL conical tube (Falcon Centrifuge Tube, Corning, Corning, NY). The residual pellet was resuspended in 1 mL of anhydrous ethanol, centrifuged (17,000 × g; 10-min; 4°C), and the supernatant was again transferred to the same 15 mL conical tube. After this rinsing process was performed a total of 3 times, the ethanol was evaporated from the 15 mL conical tube using a centrifugal concentrator (20 mbar, 248 × g, 45°C, 3 h; Vacufuge, Eppendorf, Hauppauge, NY). After evaporation, starch content was determined as previously described.

Chromium concentration of wet digesta and feces was determined by atomic absorption spectroscopy (AAnalyst 200, PerkinElmer, Waltham, MA) from supernatant after centrifugation (20,000 × g; 15-min; 4°C).

Nitrogen content of urine and dried feces was determined using the Dumas procedure (method no. 968.06; AOAC, 2012; Rapid N III, Elementar, Mt. Laurel, NJ). Plasma glucose concentrations were determined by glucose oxidase (Gochman and Schmitz, 1972).

Calculations

Digesta flow to the ileum and feces were calculated as described by Kreikemeier and Harmon (1995):

$$fluid\ flow\ (g/d) = \frac{duodenal\ Cr\ infusion\ rate\ (mg/d)}{Cr\ concentration\ of\ ileal\ or\ fecal\ fluid\ (mg/g\ DM)}$$

$$total\ digesta\ flow\ (g/d) = \frac{fluid\ flow\ (g/d)}{1 - digesta\ DM\ concentration}$$

$$DM\ flow(g/d) = total\ digesta\ flow\ (g/d) - fluid\ flow\ (g/d)$$

Ileal and fecal flow of starch or glucose were calculated as the product of DM flow and nutrient concentration. Flow of ethanol-soluble starch to the ileum or feces was calculated as fluid flow multiplied by ethanol-soluble starch concentration. Starch digestibilities in the small or large intestine were calculated as the difference of 1 and the quotient of starch flow to the end of each segment divided by starch entering each segment (Merchen, 1988).

Nitrogen balance was calculated as N intake (i.e., N intake from feed + N intake from infusate) minus N excreted (i.e., urinary N g/d + fecal N g/d) and N removed (1.23 ± 0.29 g/d) at the ileum by sampling.

Statistical analyses

Data from 1 steer receiving the control treatment in period 4 and a different steer receiving 120 g/d Glu in period 5 were missed because of failed infusions. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model statement contained treatment and period, and steer was considered a random effect. Treatment means were calculated using the LSMEANS option. Effects of Glu were determined with linear and quadratic contrasts, and contrast coefficients were calculated to account for uneven spacing of Glu treatments using Proc IML. The positive control (casein) was compared to the negative control by a *t*-test. The pH of ileal digesta and feces was analyzed as a repeated measure; treatment, period, time, and time × treatment were included in the model statement, and steer was included as a random effect. The

repeated term was time, and steer × period × treatment served as the subject. The covariance structure was autoregressive(1). Effects were considered significant at $P \leq 0.05$.

RESULTS

Small intestinal starch digestion

Nutrient flows and intestinal starch digestibilities are reported in Table 2.2. By design, amounts of starch infused at the duodenum (Table 2.2) did not differ (1,507 ± 18 g; $P \geq 0.45$). Glutamic acid infusions delivered 39.9, 80.6, and 155.5% of Glu provided by casein. Additionally, Glu infusion provided 3.35 ± 0.06, 6.76 ± 0.13, and 13.05 ± 0.37 g of N/d; casein provided 60.57 ± 2.73 g of N/d. Small intestinal starch digestion was increased 24% by casein ($P = 0.05$). Similarly, SISD increased up to 33% with greater duodenal Glu flow (*Linear* = 0.02). Increases in SISD in response to increasing flows of duodenal Glu corresponded with a decrease (*Linear* = 0.04) in ileal starch flow. Similarly, duodenal infusion of casein tended ($P = 0.07$) to decrease ileal starch flows. We observed a tendency (*Quadratic* = 0.08) for a curvilinear response among ileal glucose flow in response to increases in duodenal Glu infusion. Ileal glucose flow tended to be greatest (20 g/d) when cattle received 31 g/d duodenal Glu, but ileal glucose flow was similar to control when cattle received 62 or 120 g/d of duodenal Glu. Flows of ethanol-soluble starch were not different from control (*Linear* = 0.16; *Quadratic* = 0.30) when duodenal Glu was infused. Casein did not affect ileal flows of ethanol-soluble starch ($P = 0.42$) or glucose ($P = 0.81$). Neither Glu nor casein affected ileal pH ($P \geq$

0.48). Plasma glucose concentrations (3.35 ± 0.10 mM; Table 2.2) were not affected ($P \geq$ 0.19) by infusion of casein or Glu.

Large intestinal starch digestion

Fecal starch flow decreased 43% in response to casein ($P = 0.02$) and up to 21% with increasing amounts of Glu (*Linear* = 0.04). Casein tended ($P = 0.11$) to decrease flow of ethanol-soluble starch to feces, but had no effect ($P = 0.99$) on fecal glucose flow; however, fecal ethanol-soluble starch ($P \geq 0.69$) and glucose ($P \geq 0.65$) flows were not different when increasing amounts of Glu were infused. Large intestinal starch digestion (LISD) was not affected ($P \geq 0.17$) by increasing flow of postruminal Glu. Additionally, LISD was not affected ($P \geq 0.12$) by duodenal infusion of casein. Fecal pH did not differ despite greater duodenal casein ($P = 0.52$) or Glu ($P \geq 0.11$) flows. Differences in SISD and LISD contributed to greater postruminal starch digestion in response to greater amounts of postruminal Glu (*Linear* = 0.05) and casein ($P = 0.03$). Postruminal starch digestion increased from 70.7% to 78.2% with greater duodenal Glu flows and increased to 82.4% in response to casein.

Nitrogen balance

Nitrogen balance data are reported in Table 2.3. Greater postruminal infusion of Glu ($P \geq 0.23$) or casein ($P = 0.62$) had no impact on N intake from feed (106 ± 4.1 g/d). By design, duodenal infusion of Glu (*Linear* < 0.01) and casein ($P < 0.01$) increased N from infusate. Total N intake was not affected ($P \geq 0.58$) by increases in Glu because of variation in N intake from feed (SEM = 10.2 g/d), but total N intake was greater ($P <$

0.01) when cattle were provided duodenal casein. Because greater flows of Glu had no effect on urinary ($P \geq 0.37$) or fecal ($P \geq 0.80$) N output increases in postruminal Glu had no impact ($P \geq 0.34$) on N balance (28.9 ± 2.70 g/d). Duodenal infusion of casein (61 g N/d as casein) increased ($P < 0.01$) N excreted in urine by 33 g/d compared to control, but had no effect ($P = 0.55$) on fecal N output. Consequently, N retained was increased 22 g/d when casein was infused ($P < 0.01$); however, N balance as a percentage of N intake (26.7 ± 1.7%) did not differ when cattle received duodenal Glu ($P \geq 0.16$) or casein ($P = 0.38$).

DISCUSSION

Small intestinal starch digestion

Ostensibly, SISD provides large opportunity for improvements in energetic efficiency of dietary starch utilization in comparison to ruminal degradation. Owens et al. (1986) reviewed the available literature and reported that SISD resulted in a 42.8% improvement in feed efficiency in comparison to ruminal starch degradation. McLeod et al. (2001) observed an increase in energy retained as tissue per kcal of ME provided from starch hydrolysate abomasally infused (0.60) compared to starch hydrolysate infused in the rumen (0.48). When Branco et al. (1999) abomasally infused cattle with similar amounts of starch hydrolysate they reported that SISD was 88%. Interestingly, improvements in energetic efficiency by SISD (0.68) reported by McLeod et al. (2001) are similar (i.e., 41.7%) to improvements in feed efficiency determined by Owens et al. (1986) when the proportional amounts of energy retained as tissue per kcal of ME provided from starch hydrolysate (0.60; McLeod et al., 2001) are adjusted for amounts of

starch hydrolysate digested in the small intestine (i.e., 88%; Branco et al., 1999). Nonetheless, proportional amounts of starch able to be degraded in the rumen (>70%; Theurer, 1986) typically exceed proportional amounts of starch digested in the small intestine of cattle fed conventional diets (i.e., 52.9 ± 18.6%; Owens et al., 1986). Thus, there is typically incentive to feeding strategies that seek to maximize ruminal starch degradation because total-tract energy yield is greater (Harmon, 2009) even though starch digested in the small intestine provides nearly 42% more energy than ruminally degraded starch.

Increasing SISD to allow for greater energetic efficiency among cattle fed starch-based diets remains difficult; however, several authors have reported that greater duodenal flows of casein can improve SISD (Richards et al., 2002; Brake et al., 2014a; Brake et al., 2014b) and portal glucose absorption (Taniguchi et al., 1995). We observed greater small intestinal starch digestion when cattle received duodenal casein compared to control. Richards et al. (2002) linearly regressed SISD and small intestinal protein disappearance in steers abomasally infused with cornstarch and casein and determined that SISD was improved by 1.18 g/d for each g of protein digested in the small intestine. When amounts of starch digested in the small intestine reported by Richards et al. (2002) are regressed on amounts of casein abomasally infused, SISD was improved by 1.60 g/d for each g casein flowing to the duodenum. Similarly, Brake et al. (2014b) reported that SISD was linearly improved when cattle were provided duodenal casein in amounts up to two-fold greater than the greatest amount of casein abomasally infused by Richards et al. (2002); however, casein improved SISD at a lesser rate (0.30 g/d increase in starch digested for each g of casein duodenally infused; Brake et al., 2014b) compared to

Richards et al. (2002). Previously, we (Brake et al., 2014a) observed similar increases in SISD (0.29 and 0.34 g/d increase in starch digested per g of casein duodenally infused) in response to duodenal casein. Taniguchi et al. (1995) infused similar amounts of postruminal cornstarch and casein to those of Richards et al. (2002) and measured net glucose flux to the portal-drained viscera. These authors (Taniguchi et al., 1995) observed a 0.38 g/d increase in net glucose release to the portal-drained viscera for each g of casein infused abomasally. If the data from net portal glucose release were representative of SISD in their study (Taniguchi et al., 1995) then the findings of Taniguchi et al. (1995) are similar to measures of SISD reported by Brake et al. (2014a,b). We observed a similar rate of increase in SISD by casein (i.e., 0.30 g/d increase in cornstarch digested for each g/d of casein infused) compared to other reports (Brake et al., 2014a,b; Taniguchi et al., 1995), but less than the rate of increase in SISD by casein reported by Richards et al. (2002). Clearly, differences between experiments (e.g., site of infusion, amounts of starch and casein infused, different basal diets) may have contributed to greater increases in SISD in response to increasing duodenal casein flows reported by Richards et al. (2002) when compared to rates at which casein improved SISD reported by Brake et al. (2014a,b), Taniguchi et al. (1995), and to our current observations. Nonetheless, the average rate at which duodenal casein improved SISD across these studies (Brake et al., 2014a,b; Richards et al., 2002; Taniguchi et al., 1995) and our observations was 0.54 g/d per g/d casein flowing to the duodenum.

Brake et al. (2014a) reported that SISD was increased ($P < 0.01$) 0.96 g/d for each g of duodenal Glu when they duodenally infused 133 g/d of Glu in cattle receiving 1.4 kg/d duodenal cornstarch. Similar to the approach of Richards et al. (2002), we regressed

average amounts of starch digested in the small intestinal as a function of amounts of Glu duodenally infused:

$$y = 1.2527x + 607.79\ (r = 0.83)$$

where x is the amount of duodenal Glu infusion and y is the amount of starch digested in the small intestine. We observed linear increases of 1.25 g/d of SISD for each g of duodenal Glu infused. Rates of improvement in SISD as a function of Glu (1.25 g/d) were more than 2.3-times greater than the average rate of improvement in SISD with casein (0.54 g/d). Brake et al. (2014a) determined that AA could elicit similar improvements in SISD as casein and that Glu alone can mimic increases in SISD similar to casein. Indeed, our data indicate that duodenal Glu alone can increase SISD at rates similar to (Richards et al., 2002) or greater than (Brake et al., 2014a,b; Taniguchi et al., 1995) increases in SISD in response to casein.

Ethanol-soluble starch flow

Small intestinal starch digestion in cattle occurs in 3 steps (Huntington, 1997): 1) hydrolysis of starch by pancreatic α-amylase to oligosaccharides and limit dextrins, 2) hydrolysis of short-chain starches (i.e., oligosaccharides and limit dextrins) by membrane bound oligosaccharidases to glucose, and 3) absorption of luminal glucose.

Greater apparent flows of high-quality proteins such as casein (Swanson et al., 2002; Richards et al., 2003; Swanson et al., 2004) and soy protein (Swanson et al., 2008) to the small intestine can increase pancreatic α-amylase in cattle. Swanson et al. (2002) observed greater pancreatic α-amylase production in cattle abomasally infused with casein. Similarly, Richards et al. (2003) reported increased α-amylase secretions among

cattle postruminally infused with casein. Indeed, Harmon (2009) suggested energy as protein can augment the amylolytic capacity in the small intestine of cattle; however, it remains equivocal if α-amylase primarily limits SISD in cattle fed corn-based diets.

Several authors (Huntington, 1997; Swanson and Harmon, 2002; Harmon et al., 2004) have suggested SISD may be improved with greater α-amylase secretion in cattle. Yet, Remillard et al. (1990) found no differences in SISD when cattle were supplemented with jejunal α-amylase, indicating that a factor other than α-amylase may primarily limit SISD. Indeed, Kreikemeier and Harmon (1995) observed increased accumulation of disaccharides at the ileum among steers provided abomasal infusions of cornstarch, corn dextrins or unpolymerized glucose and concluded that the hydrolytic capacity among brush border oligosaccharidases most limited SISD in cattle.

Brake et al. (2014a) reported that duodenal infusion of Glu (133 g/d) increased SISD but not ileal ethanol-soluble starch (i.e., short-chain starch) flow in cattle. Alternatively, increases in SISD by duodenal casein tended ($P = 0.06$) to increase ileal ethanol soluble starch flow (Brake et al., 2014a). Similarly, Brake et al. (2014b) observed linear ($P = 0.01$) increases in ethanol-soluble starch flows to the ileum when up to 400 g/d casein was duodenally infused. Brake et al. (2014a) suggested that increased SISD with increased flow of short-chain starch to the ileum in response to duodenal casein may indicate greater increases in the amylolytic capacity of the small intestine than increases in hydrolytic capacity among brush border oligosaccharidases. Ethanol-soluble starch flow to the ileum was not affected by duodenal infusion of Glu or casein in this study. However, ileal ethanol-soluble starch flow numerically decreased ($Linear = 0.16$) in response to duodenal Glu and numerically increased ($P = 0.42$) in response to duodenal

casein. Numerical differences in ileal ethanol-soluble starch flows among cattle in this study are congruent with the responses reported by Brake et al. (2014a).

Glutamic acid is the primary anaplerotic substrate for enterocytes in cattle (El-Kadi et al., 2009). It is possible that greater available energy from Glu may augment hydrolytic capacity among small intestinal oligosaccharidases. Quezada-Calvillo et al. (2007) reported that increasing concentrations of short-chain oligosaccharides inhibit the hydrolytic ability of α-amylase. Therefore, increases in the hydrolytic capacity of small intestinal oligosaccharidases may allow greater overall SISD, in part, by augmenting the catalytic efficiency of α-amylase rather than increasing pancreatic α-amylase secretions, per se. Clearly, there is need for more integrated information on specific effects of increased duodenal Glu flow among α-glycohydrolases (e.g., α-amylase, oligosaccharidases) in the small intestine of cattle before a more precise understanding of the manner in which increases in SISD among cattle may be achieved.

Apparently glucose absorption did not limit SISD in our study because ileal glucose flows were small (≤ 20 g/d). Others (Kreikemeier et al., 1991; Kreikemeier and Harmon, 1995) have reported that cattle have a large capacity for small intestinal glucose absorption (i.e., 1.0 to 1.3 kg/d). Additionally, Shirazi-Beechey et al. (1991) reported that ruminants can increase glucose transport 40- to 80-fold, and that sugar substrates up-regulate intestinal transporters in ruminants. Further, when Huntington (1997) reviewed the available literature he concluded that small intestinal digestion of starch was not limited by glucose absorption.

Interestingly, we observed a tendency (*Quadratic* = 0.08) for increased ileal glucose flow when small amounts of duodenal Glu (31 g/d) were infused, but ileal

glucose flow was similar to control when greater amounts of duodenal Glu were infused. Some amino acids have been reported to antagonize glucose absorption (Hindmarsh et al., 1966); however, these authors (Hindmarsh et al., 1966) found no inhibitory effects of Glu on apparent glucose absorption among small intestinal tissue from hamsters. Additionally, ileal glucose flow was not different from control when Brake et al. (2014a) duodenally infused Glu (133 g/d) and cornstarch (1.4 kg/d) in cattle. Overall ileal glucose flows were small compared to amounts of SISD and it seems unlikely that SISD was limited by glucose absorption even though ileal glucose flow tended to be greater when the least amount of Glu was duodenally infused.

Large intestinal starch digestion

Large intestinal starch digestion was not affected by treatment despite differences among SISD in response to increases in duodenal Glu or casein. Nonetheless, increases in duodenal Glu and casein decreased fecal starch flow. When Richards et al. (2002) abomasally infused cornstarch and up to 200 g/d casein to cattle they reported that fecal starch flow was not different; however, Brake et al. (2014b) observed decreased fecal starch flow when they duodenally infused cornstarch and amounts of casein similar to amounts of casein infused in this study.

Postruminal starch digestion is the sum of starch digested in the small and large intestine. Because our treatments did not affect large intestinal starch digestion, differences in SISD were reflected in measures of postruminal starch digestion. Brake et al., (2014a,b) observed that increases in duodenal casein infusion increased postruminal starch digestion among cattle receiving duodenal starch infusions and speculated (Brake

et al., 2014a) that large intestinal starch flow may have exceeded capacity for large intestinal fermentation among cattle in their studies. We duodenally infused cattle with similar amounts of cornstarch compared to Brake et al. (2014a). It is possible that starch flow to the large intestine exceed capacity for large intestinal fermentation and contributed to a lack of response in large intestinal starch digestion to treatments. Gressley et al. (2011) reviewed the available literature and reported that an average of 87 g or 28% of starch flowing past the ileum of steers was fermented in the large intestine. Furthermore, an average of 258 g or 54% of ileal starch in dairy cattle disappeared from the large intestine. An average of 410 ± 31.5 g of starch disappeared from the large intestine among steers in this study which account for 50.4 ± 4.05% of ileal starch flow. The amount of starch digested in the large intestine in this study was greater than the average of both steers and dairy cattle reported by Gressley et al. (2011); however, the proportion of starch digested in the large intestine was similar (50.4%) to average LISD in dairy cattle (54%) reported by Gressley et al. (2011). Further, fecal N excretion was unaffected by either duodenal Glu or casein. Typically, a preponderance of N in feces is derived from microbial N (Van Soest, 1994). It is reasonable that increases in LISD that contribute to greater microbial mass in the large intestine may increase fecal N excretion. Thus, a lack of response among fecal N flow to increases in duodenal Glu or casein seem to suggest that a limit on LISD was reached in the current study. Clearly, differences in SISD can be reflected in postruminal starch digestion when LISD remains unchanged.

Interestingly, fecal pH was not affected by increases in duodenal Glu or casein despite large increases in SISD compared to control. Bissell and Hall (2010) speculated that changes in fecal pH indicated differences in SISD among cattle provided abomasal

starch infusion; however, Brake et al. (2014a) reported that SISD and fecal pH were not correlated across 2 separate experiments. Apparently, factors other than SISD can affect fecal pH in cattle.

It is possible that physical form of starch flowing to the large intestine may impact LISD. Brake et al. (2014a) observed a correlation between large intestinal starch fermentation and ileal flow of ethanol-soluble starch when they provided similar amounts of starch and casein to the duodenum, and suggested that short-chain starch may be more readily fermented in the large intestine than longer-chain starch. When cattle were provided duodenal casein, we observed a numeric increase in ileal ethanol-soluble starch flow concurrent with a numeric increase in LISD ($P = 0.11$), which might support the suggestion by Brake et al. (2014a) that short-chain starch may be more readily fermented in the large intestine of cattle than long-chain starch.

Nitrogen balance

Nitrogen provided from infusate increased with greater duodenal infusions of Glu and casein. As expected, N intake from feed did not differ because diet intake was fixed. Small variations in N intake from feed (SEM = 10.2 g/d) obviated measures of increased N intake from infusate among total N intake (infusate N + feed N) because amounts of N infused as Glu were small (i.e., 3 to 13 g/d). Conversely, duodenal infusion of casein increased total N intake because amounts of N infused as casein were 4.6-times larger than amounts of N infused as Glu. Duodenal casein increased urinary N output suggesting AA provided by casein were supplied in excess of AA requirements for protein synthesis. Increasing postruminal Glu had no effect on urinary N. Fecal N

excretion was not affected by increases in duodenal Glu or casein even though ileal starch flow was reduced in response to greater postruminal flows of Glu or casein.

Increases in duodenal Glu did not impact amounts of N retained despite linear increases in SISD; however, casein increased SISD and N retention. McLeod et al. (2001) observed a nearly 2-fold increase in energy retained in tissue among cattle infused with partially hydrolyzed starch, but N retention was only increased 32%. These authors (McLeod et al., 2001) suggested that energy available for tissue gain may have exceeded available N for lean tissue accretion. It is possible that increases in energy available for gain from increased SISD in response to greater postruminal flow of Glu are used for purposes other than protein deposition; however, it is likely that increases in energy available for gain exceeded capabilities for N deposition under conditions of our experimental model, because N retained as a proportion of N intake was not different when cattle were provided Glu or casein. Others (Reynolds et al., 2001) have reported that increases in SISD have contributed to a nearly 30% increase in amounts of N used for productive purposes. Similarly, Taniguchi et al. (1995) reported that N retention and net glucose release to the portal drained viscera were both increased nearly 50% when cattle received abomasal infusion of casein and cornstarch. Overall, these data suggest that increases in energy available for gain from greater SISD can allow for increases in lean tissue accretion.

In summary, increases in duodenal Glu linearly increased SISD at a greater rate (g/d) than casein. Additionally, numeric decreases in ileal short-chain starch flow seem to support the hypothesis that duodenal Glu may augment SISD by different mechanisms than casein. Neither increases in duodenal Glu or casein impacted LISD or fecal pH

despite altered flows of starch to the ileum. Despite observed increases in SISD postruminal Glu had little effect on N balance. Thus, additional energy from increased SISD was likely captured as fat; however, increases in N balance may have limited by protein available for greater lean tissue accretion.

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Table 2.1. Composition of soybean hull-based diet

Ingredient	% DM
Soybean hulls	72.4
Brome hay	20.0
Corn steep liquor	6.0
Limestone	1.0
Salt	0.5
Mineral and vitamin premix[1]	0.1
Chemical composition	
DM[2], %	85.0 ± 0.05
OM[2], % DM	7.7 ± 0.01
Starch[2], % DM	0.8 ± 0.04
CP[2], % DM	13.1 ± 0.01
Degradable intake protein[3], % DM	7.8
NE_m[3], Mcal/kg	1.7

[1]Provided to diet (DM basis) 100 ppm Fe, 40 ppm Mn, 60 ppm Zn, 20 ppm Cu, 1 ppm I, 0.2 ppm Se, 0.2 ppm Co, 2,200 IU of vitamin A/kg, 275 IU of vitamin D/kg, and 50 IU of vitamin E/kg.
[2]Mean ± SEM
[3]Predicted from tabular values (NRC, 2000).

Table 2.2. Effect of duodenal infusion of glutamic acid on ileal and fecal nutrient flows and small and large intestinal starch disappearance in steers receiving 1.5 kg of duodenally-infused raw cornstarch

		Glutamic acid, g/d					*P*-value		
Item	Control	31	62	120	Casein	SEM[1]	Linear	Quadratic	Control vs. casein
No. of observations	4	5	5	4	5				
Duodenal starch infused, g/d	1531	1516	1534	1479	1483	49	0.46	0.68	0.45
Ileal DM, %	15.4	14.6	15.5	14.7	14.3	0.86	0.53	0.89	0.16
Ileal pH	6.8	6.7	6.7	6.8	6.7	0.04	0.88	0.48	0.83
Nutrient flow to ileum, g/d									
DM	2716	2596	2367	2538	2681	201	0.33	0.14	0.84
Starch	965	844	801	752	801	68	0.04	0.33	0.07
Ethanol soluble starch	154	132	119	119	171	27	0.16	0.30	0.42
Glucose	3	20	14	7	1	6	0.99	0.08	0.81
Small intestinal starch digestion, %	37.2	44.4	47.9	49.6	46.1	3.3	0.02	0.16	0.05
Fecal DM, %	21.1	20.9	20.4	20.5	19.6	0.80	0.29	0.58	0.02
Fecal pH	5.5	5.6	5.6	5.5	5.6	0.08	0.17	0.11	0.52
Nutrient flow to feces, g/d									
DM	2429	2559	2280	2111	2247	283	0.28	0.75	0.59
Starch	449	523	352	356	256	67	0.04	0.83	0.02
Ethanol soluble starch	97	97	91	100	66	13.4	0.89	0.69	0.11
Glucose	43	51	42	39	43	10.7	0.65	0.72	0.99
Large intestinal starch digestion, %	52.9	37.5	56.1	60.3	67.5	7.9	0.17	0.35	0.12
Postruminal starch digestion, %	70.7	65.3	77.1	78.2	82.4	4.5	0.05	0.85	0.03
Plasma glucose, mM	3.30	3.30	3.18	3.59	3.37	0.15	0.19	0.19	0.73

[1]Largest value among treatments is reported.

Table 2.3. Effect of duodenal infusion of glutamic acid on nitrogen balance in steers receiving 1.5 kg duodenally-infused raw cornstarch

		Glutamic Acid, g/d					*P*-value		
Item, g/d	Control	31	62	120	Casein	SEM[1]	Linear	Quadratic	Control vs. Casein
Total N Intake, g/d	112	111	110	115	169	10.3	0.75	0.58	< 0.01
Feed	112	108	103	102	108	10.2	0.23	0.57	0.62
Infusate	0	3	7	13	61	1.5	< 0.01	0.95	< 0.01
Urine	30	35	33	35	63	2.6	0.37	0.50	< 0.01
Feces	47	49	47	49	49	4.1	0.80	0.80	0.55
N balance, g/d	33	27	29	31	55	6.5	0.95	0.34	< 0.01
N efficiency, % N intake	29.3	22.3	25.6	26.6	32.4	3.8	0.83	0.16	0.38

[1]Largest value among treatments is reported.

CHAPTER 3:

EFFECTS OF DIETARY FAT LEVEL AND SOURCE ON NUTRIENT DIGESTIBILITIES AND NITROGEN BALANCE IN STEERS CONSUMING CORN-BASED DIETS[1]

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[1]Contribution from the South Dakota Oil Initiative Association

ABSTRACT

Supplementing dietary lipid can augment diet energy density; however, lipids can have antimicrobial activity that can mitigate ruminal fermentation of feed. We evaluated effects of amount and source of supplementary dietary lipid on nutrient digestibility, ruminal fermentation, and N balance in cattle consuming corn-based diets. Five steers (BW = 392 ± 15 kg) fitted with ruminal, duodenal, and ileal cannulas were used in a 5 × 5 Latin square with 12-d periods. Diets contained no supplemental fat (**CON**), 4% supplemental saturated fat (tallow, **4S**), 4% supplemental unsaturated fat (linseed oil, **4U**), 8% supplemental saturated fat (tallow, **8S**), or 8% supplemental unsaturated fat (linseed oil, **8U**). Increasing amount of lipid supplementation did not affect DMI ($P \geq 0.32$); however, unsaturated lipid reduced ($P = 0.05$) DMI. Greater dietary lipid tended to decrease ruminal DM digestibility (*Linear* = 0.06) and apparent ruminal OM digestibility (*Linear* = 0.08). Increased dietary lipid tended to decrease total-tract digestibility of DM (*Linear* = 0.07), OM (*Linear* = 0.11), and NDF (*Linear* = 0.11). Additionally, unsaturated lipid tended ($P = 0.07$) to reduce total-tract NDF digestibility compared to saturated lipid. Ruminal pH was not affected by treatment ($P \geq 0.35$). Ruminal ammonia concentration tended (*Linear* = 0.15) to increase with increasing dietary lipid. Total ruminal organic acid concentration was not affected ($P \geq 0.30$) by dietary lipid. Increased dietary lipid decreased (*Linear* = 0.05) ruminal acetate concentrations and decreased (*Linear* = 0.01) the ratio of acetate to propionate. Ruminal acetate ($P = 0.07$), propionate ($P = 0.06$), and acetate:propionate ($P = 0.02$) tended to be affected by the interaction of source × level. Microbial N flow to the duodenum was not affected ($P \geq 0.17$) by lipid source or level, but microbial efficiency was linearly ($P = 0.05$) increased with increased

level of dietary lipid. Unsaturated lipid decreased ($P = 0.02$) urinary N. Urinary N was not different ($P \geq 0.51$) with increasing lipid, but tended ($P = 0.12$) to be affected by the interaction of source × level. Fecal N tended (*Linear* = 0.15) to decrease in response to greater dietary lipid and was reduced ($P < 0.01$) by unsaturated lipid. Despite altered urine and fecal N outputs, small variations in N intake, urinary N, and fecal N mitigated responses among N balance. N efficiency was not affected ($P \geq 0.18$) by lipid source or amount.

Key Words: cattle, lipid, nitrogen balance, nutrient digestibility

INTRODUCTION

Cattle performance is often first limited by energy available for productive purposes (Lofgreen and Garrett, 1968; NRC, 2000). Supplementation of cattle diets with lipids is an effective way to increase diet energy density; however, lipids can have deleterious effects on ruminal bacteria (Jenkins, 1993; Zinn et al., 1994; Hess et al., 2008). Ostensibly, dietary lipid has greater impact among fibrolytic bacteria because fiber fermentation is typically reduced with addition of dietary lipid (Zinn et al., 1994). Effects of lipid appear to differ between lipid source. Indeed, unsaturated lipids apparently have greater impact on ruminal bacteria than saturated lipids (Hess et al., 2008). Antimicrobial effects of lipids have opportunity to have substantial impacts on metabolizable nutrient flow and subsequently growth and performance of ruminants. Production of microbial protein by ruminal bacteria is responsible for a large proportion of duodenal MP flow (NRC, 2000), and energetic end-products of ruminal fermentation typically provide the greatest amount of net energy to cattle (Huntington, 1997). It is possible that a decrease in duodenal MP flow and reduced VFA production would lead to a decrease in N balance. Further, there is a paucity of data regarding the effects of supplemental dietary lipid on N balance in cattle. The objectives of this study were to determine the effect of amount and source of lipid supplementation on nutrient digestibility, ruminal fermentation characteristics, and nitrogen balance in cattle.

MATERIALS AND METHODS

Animals and diets

All experimental protocols and animal husbandry procedures were approved by the South Dakota Institutional Animal Care and Use Committee. Five ruminally, duodenally, and ileally cannulated steers (BW = 392 ± 15 kg) were allowed *ad libitum* access to water and housed in individual pens (1.7 × 2.4 m) in a temperature controlled room (21°C) under 16 h of light (0500 to 2100 h) and 8 h of darkness. Cattle were placed in a 5 × 5 Latin square; each period consisted of 7 d of adaptation and samples were collected during the subsequent 5 d.

Treatments consisted of 5 corn-based diets (Table 3.1) of varying fat source (saturated or unsaturated fat) and supplementation amount (4 or 8% added fat). Treatments were designed to deliver no additional fat (**CON**), 4% (**4S)** or 8% (**8S**) additional lipid from tallow, and 4% (**4U**) or 8% (**8U**) additional lipid from linseed oil. The fatty acid composition of diets is shown in Table 3.2. Diets were offered (DM basis) at 105% of the average DMI over the previous 4 d and were fed in equal amounts every 12 h (0700 and 1900 h). Five g of TiO_2 was added to each 12-h feed offering as an indigestible nutrient flow marker beginning on d 5 of each period and continuing through the end of each period.

A temporary indwelling guidewire-style catheter (MILACath, Mila International, Inc., Erlanger, KY) was placed into a jugular vein of each animal on d 8 of each period for infusion of $^{15}N^{15}N$-urea. Each period, immediately prior to the initial infusion, infusate was prepared in a laminar-flow hood using sterile techniques by combining 3.6 g

$^{15}N^{15}N$-urea (98 APE, 99%, Sigma-Aldrich, Inc., St. Louis, MO) per L of sterile saline solution (0.9% NaCl). The infusate was filtered through a 0.22-µm filter (Sterivex, Millipore Corporation, Billerica, MA) into sterilized glass bottles and sealed with a rubber septum and cap. Infusate was stored at 4°C until use.

Sterile saline was continuously infused immediately after catheter placement until 1900 h on d 9 at which point cattle were continuously infused (4.16 ml/h) with $^{15}N^{15}N$-urea solution until the end of the period. Infusions were delivered via a programmable syringe pump (PHD Ultra, Harvard Apparatus, Holliston, MA) and delivered at a rate 0.24 mmol/h.

Measurements

Cattle were moved to metabolism stalls on d 8 to facilitate total collection of urine and feces from day 8 to 11 for measure of N balance. Diet (100 g/d) and ort samples (10%, if present) were collected from d 7 to 10 to correspond to urine and fecal samples collected from d 8 to 11. Feces was collected in pans located behind cattle and 5% of the total output was composited daily and frozen (-20°C). Urine was collected daily into a clean vessel containing 900 mL of 10% (wt/wt) H_2SO_4, and 1% of the total output was composited and frozen (-20°C). Jugular blood was collected into heparinized (143 USP) tubes (16 × 100 mm; Benton Dickinson, Franklin Lakes, NJ) via venipuncture 12 h after feeding on d 11 for measures of PUN and plasma glucose. Blood tubes were immediately placed on ice and plasma was harvested after centrifugation (2,200 × g; 15-min; 4°C) and frozen (-20°C).

On d 12 of each period, ruminal, duodenal, and ileal samples were collected every 2 h across a 12-h (0700 to 1900 h) period. Ruminal samples were collected after manually mixing rumen contents by hand. An aliquot (~ 400 mL) of ruminal contents was removed and strained through 4 layers of cheesecloth. Ruminal fluid pH was immediately measured with a pH meter (Orion 3 Star, Thermo Scientific, Waltham, MA) calibrated at 3 points (i.e., 4.0, 7.0, and 10.0). An 8 mL aliquot of strained rumen fluid was mixed with 2 mL 25% (wt/vol) m-phosphoric acid for subsequent determination of VFA and lactic acid. Another aliquot of 10 mL strained rumen fluid was mixed with 1 mL 6 M HCl for ammonia analysis. The remaining strained ruminal fluid was recombined with the previously filtered particulate and 450 mL 0.9% saline and blended (Model CB15V, Waring Commercial, Torrington, CT) for 1 min to dissociate ruminal bacteria from particulate. Blended ruminal contents and saline were then strained through the same 4 layers of cheese cloth and, 150 mL was composited and frozen (-20°C). Duodenal (222 ± 4 g) and ileal (84 ± 5 g) digesta samples were collected by attaching a plastic bag (140 × 229 mm, Fisherbrand, Fisher Scientific) to each cannula for 1 h. Duodenal and ileal pH was immediately measured with the same pH meter, composited, and frozen (-20°C).

Laboratory analyses

Diet and ort composite subsamples were dried in a forced air oven at 55°C for 24 h, air equilibrated and weighed to determine partial DM. Ruminal, duodenal, ileal and fecal samples were freeze-dried. Once dried, all samples were ground to pass a 1-mm screen (Thomas-Wiley Laboratory Mill Model 4, Thomas Scientific USA, Swedesboro,

NJ). The DM of feed, ort, duodenal, ileal, and fecal samples was determined by drying for 24 h at 105°C in a forced-air oven (method no. 934.01; AOAC 2012). The OM was determined by ashing for 8 h in a muffle furnace at 500°C.

Nitrogen content of feed, ort, duodenal, ileal, fecal and urine samples was determined using the Dumas procedure (AOAC 968.06; Rapid N III, Elementar, Mt. Laurel, NJ). Duodenal and fecal TiO_2 concentrations were determined colorimetrically after preparation of samples as described by Leone (1973). Dried diet samples were analyzed by gas chromatography for LCFA using the procedures of Sukhija and Palmquist (1988). Duodenal and fecal samples were analyzed for NDF (Ankom-Fiber Analyzer 200, Ankom Technology, Fairport, NY; with amylase and without ash correction) according to the method of Van Soest et al. (1991).

Ruminal bacteria were isolated by thawing samples of ruminal contents and then centrifuging samples at 500 × *g* for 20 min. Supernatant was then centrifuged at 20,000 × *g* for 20 min to form a bacterial pellet. The pellet was resuspended with saline (0.9% NaCl), centrifuged again at 20,000 × *g* for 20 min, frozen, and freeze-dried. Dried bacterial, duodenal, and fecal samples were analyzed for ^{15}N enrichment via stable isotope mass spectroscopy. Ruminal VFA were determined by GC as described by Vanzant and Cochran (1994), and lactic acid concentrations were determined via colorimetric assay as described by Barker and Summerson (1941). Ruminal ammonia (Broderick and Kang, 1980) and plasma urea (Marsh et al., 1965) were measured colorimetrically.

Starch concentrations of feed, orts, duodenal digesta and feces were determined using the procedures of Herrera-Saldana and Huber (1989) with glucose measurement

according to Gochman and Schmitz (1972). Plasma glucose concentrations were also analyzed via glucose analysis (Gochman and Schmitz, 1972).

Calculations

Duodenal and ileal flows were calculated as the fecal output of TiO_2 divided by the TiO_2 concentration of digesta. Bacterial and duodenal ^{15}N enrichments were calculated as ^{15}N/total N. Bacterial N flow was calculated by multiplying duodenal N flow by the ratio of duodenal ^{15}N enrichment to bacterial ^{15}N enrichment. Duodenal flow of ruminally undegraded N from feed was calculated by subtracting microbial N flow from total duodenal N flow. Nutrient digestibilities were calculated as the difference of 1 and the quotient of nutrient flow to the end of each segment divided by nutrient entering each segment. Apparent ruminal OM digestibility was corrected for flow of bacterial OM to calculate true ruminal OM digestibility. Microbial efficiency was calculated as the quotient of duodenal microbial N flow divided by ruminal truly digested OM. Nitrogen balance was calculated as N intake from feed minus N excreted (i.e., urinary N g/d + fecal N g/d). Nitrogen efficiency was calculated by dividing N balance (g/d) by N intake (g/d).

Statistical analyses

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). Data from 1 steer in 1 period receiving 4% linseed oil were removed for ruminal and postruminal digestibility calculations due to apparent marker failure. For microbial N, UIP, and microbial efficiency calculations, data from 2 steers in 1 period

and 1 steer in another period due to apparent marker failure. For variables without repeated sampling, terms in the model included treatment and period with steer included as a random effect. Model terms for fermentation profile variables were treatment, period, hour, and hour × treatment with steer included as a random term. The repeated term was hour, with steer × period serving as the subject. Autoregressive(1) was used for the covariance structure. The LSMEANS option was used to calculate treatment means. Effects of lipid addition were determined with linear and quadratic contrasts. Preplanned contrasts of lipid source and the interaction of lipid source × lipid amount were also evaluated. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.15$.

RESULTS AND DISCUSSION

Nutrient intake and digestibility

Nutrient intake and digestibility data are presented in Table 3.3. By design, lipid intake increased with increasing dietary lipid content (*Linear* < 0.01). Dry matter intake did not differ ($P \geq 0.32$) with increasing dietary lipid content, but decreased ($P = 0.05$) with unsaturated lipid compared to saturated lipid. Similarly, OM ($P = 0.05$) and NDF ($P = 0.01$) intake was reduced when cattle were fed diets with unsaturated lipid. Starch intake decreased (*Linear* = 0.03) with increasing lipid and tended ($P = 0.08$) to decrease in response to unsaturated lipid. Lipid supplementation to both forage- (Pavan et al., 2007; Martin et al., 2008) and corn-based (Zinn and Plascencia, 2004) diets has reduced DMI when fed at concentrations greater than 6% of DM. Pavan et al. (2007) reported a linear decrease of 23% in DMI when cattle grazing pasture were supplemented with up to

1.5 g of corn oil per kg of BW. Martin et al. (2008) used linseed oil to increase dietary lipid in corn silage-based diets to 8% and observed a 26% decrease in DMI. Additionally, Zinn and Plascencia (2004) reported a 13% decrease in DMI when the dietary lipid content was increased up to 11% for cattle consuming a corn-based diet. This decrease in DMI with large amounts of lipid supplementation likely results from increased energy density of diets, but may also be related to decreased ruminal digestibility of other feeds; however, it is unlikely that physical fill limited intake among our diets.

Lipid supplementation has been shown to diminish ruminal fermentation of other dietary energy substrates in cattle (Jenkins, 1993; Doreau and Chilliard, 1997). Ruminal fiber digestion is especially impacted by dietary lipid supplementation. The addition of 10% lipid to the diet of cattle can disrupt ruminal structural carbohydrate fermentation by over 50% (Jenkins, 1993). This reduction in fiber fermentation can reduce VFA production in the rumen, and therefore reduce the capture of energy from nonstructural carbohydrates. We observed a tendency for decreased (*Linear* = 0.06) ruminal DM digestibility but no change (75.9 ± 3.19%; $P \geq 0.37$) in ruminal NDF digestibility in response to dietary lipid level. Negative disruptions in fiber digestion typically occur when lipids make up greater than 5% of the diet DM (Doreau and Chilliard, 1997). Indeed, Plascencia et al. (2012) observed no change in ruminal NDF digestion when they supplemented steers with 3% yellow grease. Similarly, Elliott et al. (1997) fed up to 5.7% fat diets to steers and found no differences in NDF digestibility compared to control. Nonetheless, Oldick and Firkins (2000) supplemented corn silage-based diets with an additional 5% lipids of varying degrees of unsaturation and observed reduced ruminal NDF digestion with lipid addition; however, degree of unsaturation had no effect. We

observed no effect ($P = 0.87$) among ruminal NDF digestibility in response to unsaturated lipids. Lipids with a greater degree of unsaturation generally have a greater effect on ruminal fermentation of fiber (Jenkins, 1993). Hristov et al. (2005) demonstrated that more unsaturated (linoleic) oils could depress ruminal NDF digestion to a greater degree than more saturated (oleic) oils in steers consuming concentrate-based diets. These data in conjunction with our observations indicate that increased levels of dietary lipid, and especially highly unsaturated lipid sources, can diminish fiber digestion in the rumen of cattle.

Ruminal starch digestion is typically not reduced by addition of lipid to diets (Jenkins, 1993; Zinn et al., 1994). Starch digestion in the rumen was unaffected by lipid amount or source (79.2 ± 3.28%; $P \geq 0.39$). Similarly, Avila et al. (2000) measured ruminal starch digestion in lactating cattle fed diets containing 6.5% DM as lipids of differing degree of unsaturation and found no effect of either fat source or inclusion level on ruminal starch digestion. However, Plascencia et al. (2012) reported slight decreases in ruminal starch fermentation when yellow grease was added to dry rolled corn-based diets, but not when yellow grease was added to steam flaked corn-based diets.

Organic matter truly fermented in the rumen was unchanged when we fed cattle greater amounts of lipid ($P \geq 0.41$); however, apparent ruminal OM digestibility tended (*Linear* = 0.08) to decrease. Others (Oldick and Firkins, 2000) have reported no effect of increased fat supplementation on apparent or true OM digestibility. When Scholljegerdes et al. (2004) supplemented cattle with lipids of varying levels of unsaturation, they observed a decrease in truly fermentable OM with increasing fat supplementation, with no effect of degree of unsaturation. Additionally, Elliott et al. (1997) detected a

depression in apparent OM digestibility in the rumen of steers fed diets up to 5.7% fat. A reduction in OM can often be attributed to lipids being indigestible in the rumen (Zinn et al., 1994). When rumen undegradable lipids replace readily fermented energy sources in most studies, the proportion of potentially fermentable OM is inherently decreased. Reduced OM digestibility can also be attributed to reduced fiber digestion in response to greater dietary lipid content. Interestingly, reductions in ruminal fiber fermentation in response to greater dietary lipid do not generally mitigate duodenal flow of microbial N (Oldick and Firkins, 2000; Gozho et al., 2008; Mutsvangwa et al., 2012).

Microbial N flow to the duodenum is often unaffected by lipid supplementation (Oldick and Firkins, 2000; Gozho et al., 2008; Mutsvangwa et al., 2012). This, in combination with a reduction in OM digestibility often observed with greater dietary lipid inclusion can lead to greater microbial efficiency (g microbial N/kg OM truly fermented). Microbial N flow (Table 3.4) was unaffected (75.6 ± 9.27 g/d; $P \geq 0.16$) in response to amount or source of lipid. Nonetheless, variation in measures of microbial N flows and true ruminal OM digestibility resulted in a more than 2-fold increase (*Linear* = 0.05) in microbial efficiency in response to greater dietary lipid. Lipid source did not affect (P = 0.95) microbial efficiency. Oldick and Firkins (2000) reported a tendency (P = 0.07) for microbial efficiency to increase with greater fat supplementation. Conversely, others (Mutsvangwa et al., 2012) have also reported no change in microbial efficiency among cattle consuming diets containing 5% lipid of various degree of unsaturation. Additionally, lipid source did not affect (P = 0.95) duodenal UIP flows; however, there was a slight tendency (*Linear* = 0.15) for increased UIP with increased dietary lipid content.

Total-tract digestibility of DM (*Linear* = 0.07) and OM (*Linear* = 0.11) tended to decrease with greater levels of dietary lipid, but were not different ($P \geq 0.36$) in response to lipid source. When Górka et al. (2015) fed increasing concentrations of lipids to cattle, total tract digestibility of OM was linearly decreased. Elliott et al. (1997) detected decreased total tract OM digestion in response to both increased level of fat supplementation and degree of unsaturation of lipid in corn silage-based diets. Similar to our observations, Avila et al. (2000) observed no differences in total tract digestibilities in response to degree of unsaturation of dietary lipids. We also observed a tendency for decreased total-tract NDF digestibility in response to source ($P = 0.07$) and level (*Linear* = 0.11) of dietary lipid. Total-tract starch digestion was not different ($P \geq 0.42$) among treatments. Others (Zinn, 1989; Plascencia et al., 2012) have similarly reported no change among total-tract starch digestibility in response to the addition of dietary lipid.

Reports on effects of lipid amount and source on total-tract digestion of nutrients among ruminants are limited. It appears that source and level of dietary fat inclusion may influence the total-tract digestibilities of DM, OM, and NDF. However, the total-tract digestibility of starch seems to be unaffected.

Rumen fermentation characteristics

Amount or source of lipid supplementation had no effect on ruminal pH (6.02; $P \geq 0.35$; Figure 3.1). Similarly, no change in ruminal pH was observed when Lima et al. (2014) dosed 200 g/d soybean oil directly into the rumen of cattle. Ruminal pH was not affected in cattle fed steam-rolled barley diets with up to 10% dietary lipid (Zinn, 1989). Conversely, Górka et al. (2015) and Elliott et al. (1997) reported increases in mean rumen

pH as dietary fat increased up to 7.7 or 5.6% of diet DM, respectively. These shifts in ruminal pH could be due in part to decreased fermentable carbohydrates available to the rumen microbes as increases in dietary lipid replaced corn.

Total organic acid concentration (Table 3.4) was not affected by lipid source (P = 0.61) or level ($P \geq 0.30$). Elliott et al. (1997) reported decreased total VFA concentrations when cattle were supplemented with lipid, however, lipid saturation level had no effect. Further, these authors (Elliott et al., 1997) observed similar decreases in ruminal acetate concentration and increases in ruminal propionate concentrations with greater lipid supplementation. Elliott et al. (1997) suggested that increases in ruminal pH and reduced VFA production could be related to reductions in fermentation of starch because lipid replaced corn in their diets. However, others (Avila et al., 2000; Scholljegerdes et al., 2004) have reported no change in rumen pH or VFA concentration when lipids were supplemented.

We observed a decrease (*Linear* = 0.05) in ruminal acetate and the ratio of acetate:propionate (*Linear* = 0.01) with increases in diet lipid content. Interestingly, acetate (P = 0.07) and propionate (P = 0.06) tended to be affected by the interaction of source × level, thus the ratio of acetate:propionate was also affected (P = 0.02) by the interaction.

Rumen ammonia tended to increase (*Linear* = 0.15; Figure 3.2) in response to greater dietary lipid in our study. Similarly, Górka et al. (2015) observed increased ruminal ammonia concentrations with greater dietary lipid. Others (Elliott et al., 1997; Avila et al., 2000; Lima et al., 2014) have reported no change in rumen ammonia when lipids were supplemented. However, ammonia is a key source of N for microbial protein

synthesis. When fiber digestion is inhibited by high amounts of lipid supplementation, microbial protein synthesis is subsequently reduced, thereby reducing demand for ruminal ammonia. Thus, it is logical that increased lipid supplementation could increase ruminal ammonia concentrations.

Nitrogen balance

Nitrogen balance data is presented in Table 3.5. Our diets were designed to be isonitrogenous, and N intake from feed did not differ among treatments (138.6 ± 9.00 g/d; $P \geq 0.24$). Urinary N excretion decreased ($P = 0.02$) for steers fed unsaturated lipids (50.9 g/d) compared to those fed saturated lipid (56.9 g/d). Similarly, fecal N excretion was reduced ($P < 0.01$) in cattle fed unsaturated lipid (35.1 g/d) compared to when cattle were fed saturated lipid (46.6 g/d). Despite differences in urinary and fecal N excretion, N balance was not affected by lipid supplementation source or level (43.0 ± 4.78 g/d; $P \geq 0.37$).

Limited data exists for effects of lipid supplementation level on N balance in cattle. Gozho et al. (2008) observed no differences in either tissue N retention (30.9 g/d) or milk N (152.0 g/d) in response to lipid source when cattle consumed diets with either whole canola (i.e., a more saturated lipid) or flax (i.e., a more unsaturated lipid), resulting in total productive N of 182.9 g/d. The tissue N balance reported by Gozho et al. (2008) was less than our observations (43.0 g/d), but this disparity could be attributed to the differences in physiologic maturity of the cattle used in each study. Overall, the efficiency of productive N use observed by Gozho et al. (2008) was 30.5%. We did not observe any effects of lipid source or level on N efficiency (28.5 ± 2.70%; $P \geq 0.18$) in

the current study. Additionally, Benchaar et al. (2015) reported an average productive N efficiency of 37.0% resulted from no change in tissue N balance or productive N when cattle were fed 6% dietary lipid in silage-based diets with linseed oil.

Plasma metabolite data is presented in Table 3.5. Plasma urea-N (PUN) was unaffected by amount or source of lipid (4.80 ± 0.52 mM; $P \geq 0.48$). Similarly, plasma glucose was not different (3.28 ± 0.07; $P \geq 0.51$) among treatments. Avila et al. (2000) measured blood metabolites in cattle fed forage-based diets containing 6.5% lipid and found no change in PUN, but a tendency ($P = 0.07$) for decreased plasma glucose compared to control cattle. No change in PUN was observed when Gozho et al. (2008) fed silage-based diets containing 5.3% of diet DM as lipid of varying degree of unsaturation.

Greater dietary fat content can increase the proportion of fat to lean tissue in beef carcasses (Doreau and Chilliard, 1997). Zinn et al. (1994) fed cattle increasing amounts of dietary fat and reported a 13% decrease in ADG but no change in HCW when up to 14% of the diet DM was lipid. Conversely, Pavan et al. (2007) observed a tendency ($P = 0.09$) for increased ADG and a linear increase in HCW when they fed greater amounts of corn oil to steers. When cattle consumed diets differing in lipid saturation level, Felton and Kerley (2004) found no effect of lipid saturation on carcass characteristics.

In summary, greater dietary lipid content can reduce ruminal fermentation of OM in cattle. Reductions in total-tract digestion of DM, OM, and NDF can also result from increased dietary lipid amount or source. Additionally, greater dietary lipid can improve the efficiency of microbial N capture. Lipid amount and source can affect molar proportions of acetate and shift the ratio of acetate:propionate in the rumen without

affecting the total concentration of organic acids. Amount and degree of unsaturation among dietary lipids can affect urine and fecal N output; however, N balance and N efficiency were not different.

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Table 3.1. Composition of dry rolled corn-based diets

	Dietary treatment[1]				
Item, % DM	Control	4S	4U	8S	8U
Ingredient					
DRC	67.4	62.2	62.2	57.0	57.0
Alfalfa hay	10.0	10.0	10.0	10.0	10.0
Linseed meal	10.8	12.0	12.0	13.2	13.2
Linseed oil	0.0	0.0	4.0	0.0	8.0
Tallow	0.0	4.0	0.0	8.0	0.0
Cane molasses, liquid	10.0	10.0	10.0	10.0	10.0
Limestone	1.2	1.2	1.2	1.2	1.2
Salt	0.5	0.5	0.5	0.5	0.5
Mineral mix[2]	0.1	0.1	0.1	0.1	0.1
Chemical composition					
OM	93.69	93.90	93.40	93.53	93.54
N	2.28	2.23	2.43	2.29	2.35
DIP[3]	7.72	7.81	7.81	7.90	7.90
Starch	36.46	33.77	32.48	31.26	33.46
NDF	19.4	18.7	18.0	20.9	18.7
Total fatty acids	3.52	6.83	7.20	10.14	10.88
NE_m, Mcal/kg[3]	1.98	2.09	2.09	2.21	2.21

[1]Treatments delivered no additional fat (**CON**), 4% (**4S)** or 8% (**8S**) additional fat from tallow, or 4% (**4U**) or 8% (**8U**) additional fat from linseed oil.

[2]Designed to provide to diet (DM basis) 100 ppm Fe, 40 ppm Mn, 60 ppm Zn, 20 ppm Cu, 1 ppm I, 0.2 ppm Se, 0.2 ppm Co, 2,200 IU of vitamin A/kg, 275 IU of vitamin D/kg, and 50 IU of vitamin E/kg.

[3]Predicted from tabular values (NRC, 2000).

Table 3.2. Fatty acid composition of dry-rolled corn-based diets.

	Dietary treatment[1]				
Item	Control	4S	4U	8S	8U
FA, % of total					
$C_{10:0}$	0.11	0.09	0.05	0.08	0.03
$C_{12:0}$	0.21	0.15	0.11	0.13	0.07
$C_{14:0}$	0.16	1.85	0.11	2.43	0.09
$C_{16:0}$	14.96	20.02	9.73	21.77	8.03
$C_{16:1}$	0.19	1.36	0.13	1.77	0.11
$C_{18:0}$	2.12	11.35	3.01	14.55	3.30
$C_{18:1}$	25.90	33.99	23.69	36.79	22.98
$C_{18:2}$	50.17	25.84	30.72	17.41	24.43
$C_{18:3}$	4.08	2.26	31.00	1.63	39.71
$C_{20:0}$	0.52	0.33	0.33	0.27	0.26
$C_{20:1}$	0.31	0.26	0.17	0.24	0.12
$C_{20:2}$	0.08	0.08	0.06	0.08	0.06
$C_{22:0}$	0.34	0.18	0.25	0.13	0.22
$C_{22:1}$	0.05	0.03	0.04	0.03	0.04
$C_{24:0}$	0.37	0.20	0.24	0.14	0.20
SFA	18.79	34.16	13.81	39.50	12.21
MUFA	26.46	35.64	24.03	38.82	23.25
PUFA	54.32	28.18	61.78	19.11	64.20
FA intake, g/d					
$C_{10:0}$	0.23	0.39	0.20	0.49	0.20
$C_{12:0}$	0.47	0.68	0.42	0.80	0.41
$C_{14:0}$	0.36	8.33	0.43	15.07	0.53
$C_{16:0}$	33.06	90.27	38.81	134.99	47.24
$C_{16:1}$	0.43	6.14	0.51	10.96	0.63
$C_{18:0}$	4.69	51.18	12.01	90.20	19.38
$C_{18:1}$	57.23	153.28	94.54	228.11	135.13
$C_{18:2}$	110.87	116.56	122.57	107.94	143.66
$C_{18:3}$	9.02	10.18	123.69	10.08	233.48
$C_{20:0}$	1.15	1.50	1.30	1.67	1.54
$C_{20:1}$	0.69	1.17	0.66	1.48	0.69
$C_{20:2}$	0.17	0.35	0.25	0.48	0.35
$C_{22:0}$	0.74	0.83	0.99	0.82	1.29
$C_{22:1}$	0.11	0.15	0.18	0.16	0.25
$C_{24:0}$	0.82	0.89	0.97	0.85	1.18
SFA	42	154	55	245	72
MUFA	58	161	96	241	137
PUFA	120	127	247	118	377

[1]Treatments delivered no additional fat (**CON**), 4% (**4S)** or 8% (**8S**) additional fat from tallow, or 4% (**4U**) or 8% (**8U**) additional fat from linseed oil.

Table 3.3. Effect of supplemental fat source and level on intake and nutrient digestibility in steers consuming a corn-based diet.

	Dietary treatment[1]						*P*-value			
								Level		
Item,	CON	4S	4U	8S	8U	SEM[2]	Source	Linear	Quadratic	Source*Level
Intake, kg/d										
DM	6.29	6.60	5.54	6.11	5.41	0.911	0.05	0.32	0.90	0.67
OM	5.89	6.20	5.17	5.72	5.06	0.853	0.05	0.31	0.89	0.64
NDF	1.22	1.23	1.00	1.27	1.01	0.176	0.01	0.47	0.41	0.87
Starch	2.29	2.23	1.80	1.91	1.81	0.306	0.08	0.03	0.64	0.26
Lipid	0.22	0.45	0.40	0.62	0.59	0.069	0.26	<0.01	0.72	0.78
Ruminal digestion[3], %										
DM	68.9	56.3	69.0	44.5	47.4	11.71	0.42	0.06	0.56	0.60
OM, apparent	72.6	61.7	72.1	58.5	57.2	8.20	0.50	0.08	0.79	0.39
OM, true	82.9	83.8	100.3	74.7	90.5	13.59	0.19	0.98	0.41	0.98
NDF	81.7	71.5	82.6	77.4	68.9	8.72	0.87	0.37	0.96	0.24
Starch	82.2	82.4	80.4	74.3	75.3	8.70	0.94	0.39	0.67	0.84
Total-tract digestion, %										
DM	79.9	77.7	80.2	76.3	76.5	1.48	0.36	0.07	0.56	0.43
OM	80.6	78.5	81.0	77.4	77.4	1.49	0.40	0.11	0.58	0.42
NDF	73.6	69.7	67.5	72.1	65.6	2.26	0.07	0.11	0.24	0.37
Starch	90.2	88.2	90.3	88.8	87.8	1.90	0.78	0.42	0.99	0.43

[1]Treatments delivered no additional fat (**CON**), 4% (**4S)** or 8% (**8S**) additional fat from tallow, or 4% (**4U**) or 8% (**8U**) additional fat from linseed oil.

[2]Largest value among treatments is reported.

[3]Data from 1 steer in 1 period were removed due to apparent marker failure.

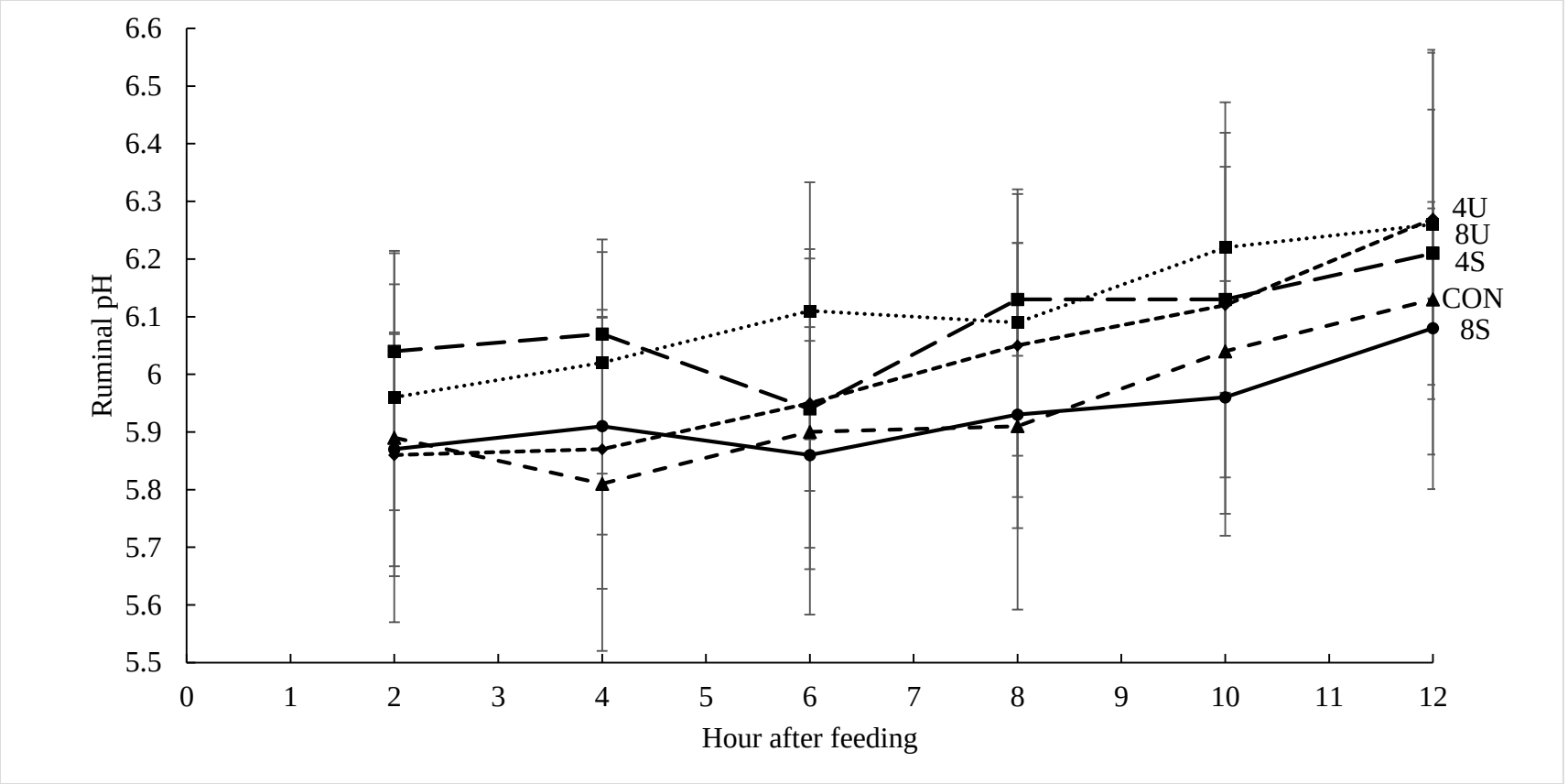

Figure 3.1. Effect of dietary lipid content and source on ruminal pH in cattle consuming corn-based diets. Treatment, $P = 0.83$. Treatment × h, $P = 0.11$.

Table 3.4. Ruminal fermentation characteristics and duodenal protein flow in steers fed different amounts and sources of dietary lipid

	Dietary treatment[1]						*P*-value			
								Level		
Item,	CON	4S	4U	8S	8U	SEM	Source	Linear	Quadratic	Source*Level
Total organic acids, mM	103.4	96.4	103.7	101.3	98.8	13.15	0.61	0.57	0.71	0.30
Acetate, mM	52.86	50.11	51.59	50.36	41.90	5.014	0.21	0.05	0.60	0.07
Propionate, mM	25.35	28.40	26.91	25.63	30.72	4.061	0.31	0.19	0.59	0.06
Butyrate, mM	16.82	11.68	18.17	18.49	18.80	5.085	0.33	0.67	0.39	0.38
Isobutyrate, mM	1.26	1.18	1.28	1.29	1.28	0.195	0.61	0.84	0.67	0.61
Valerate, mM	3.36	2.22	2.03	2.23	2.47	0.822	0.95	0.07	0.08	0.63
Isovalerate, mM	3.61	2.73	3.64	3.21	3.54	0.566	0.11	0.62	0.40	0.46
Lactate, mM	0.08	0.09	0.10	0.09	0.09	0.012	0.34	0.49	0.67	0.62
A:P	2.38	1.78	2.16	2.03	1.50	0.235	0.71	0.01	0.57	0.02
Duodenal flow, g/d										
Microbial N[2]	57.4	97.6	40.2	84.8	90.2	26.41	0.19	0.16	0.85	0.12
UIP[2]	40.2	58.7	63.1	60.0	89.8	27.68	0.43	0.15	0.88	0.55
Microbial efficiency[2], %	11.5	20.8	13.2	23.0	29.8	8.78	0.95	0.05	0.77	0.28

[1]Treatments delivered no additional fat (**CON**), 4% (**4S)** or 8% (**8S**) additional fat from tallow, or 4% (**4U**) or 8% (**8U**) additional fat from linseed oil.

[2]Data from 2 steers in 1 period and 1 steer in another period removed due to apparent marker failure.

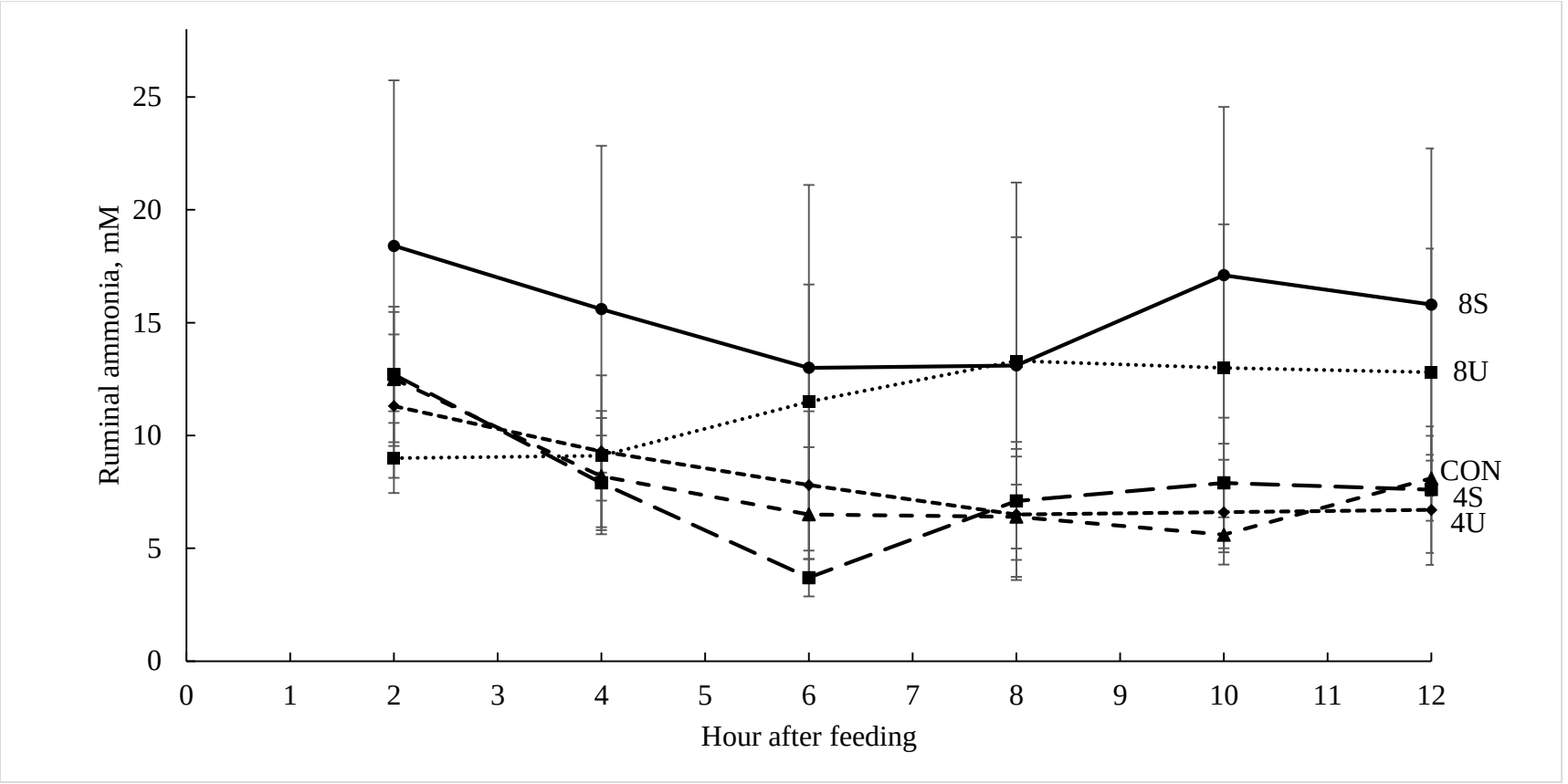

Figure 3.2. Effect of dietary lipid content and source on ruminal ammonia concentration in cattle consuming corn-based diets.

Treatment, $P = 0.33$. Treatment × h, $P = 0.44$.

Table 3.5. Nitrogen balance and plasma metabolites in steers fed differing amounts and sources of lipid.

	Dietary treatment[1]						*P*-value			
								Level		
Item,	CON	4S	4U	8S	8U	SEM	Source	Linear	Quadratic	Source*Level
Feed N intake, g	143.0	145.4	136.4	141.0	127.3	20.94	0.24	0.45	0.79	0.80
Urinary N, g	55.8	58.9	49.0	55.0	52.8	5.39	0.02	0.51	0.69	0.12
Fecal N, g	44.3	48.5	36.5	44.7	33.8	6.98	< 0.01	0.15	0.78	0.85
N balance, g	43.0	38.2	51.2	41.5	41.0	11.40	0.41	0.85	0.72	0.37
N efficiency, %	26.8	23.1	34.0	27.2	31.6	6.31	0.18	0.70	0.93	0.56
Plasma										
Urea-N, mM	4.78	3.75	5.74	4.94	4.81	1.280	0.48	0.96	0.94	0.42
Glucose, mM	3.28	3.33	3.19	3.27	3.32	0.158	0.75	0.90	0.85	0.51

[1]Treatments delivered no additional fat (**CON**), 4% (**4S)** or 8% (**8S**) additional fat from tallow, or 4% (**4U**) or 8% (**8U**) additional fat from linseed oil.